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Editorial

Anyone for a symposium?

Time was when symposium was not a portentous word for a ragbag of second rate papers artfully salted with a few "names." Time was when a medical symposium meant a series of learned and unbiased presentations by experts in the course of which the controversial issues were sought out, exposed, and argued. Time was when a symposium, like so many of the McCollum-Pratt Symposia held under the auspices of the Johns Hopkins University, was an experience to be remembered. Time was when a symposium was like an exciting dinner party with good food and carefully chosen guests sparking each other off into brilliant discussion. Time was when a published symposium was likely to be worth buying, reading, and keeping. This was not so long ago—but times have changed.

This is not to say that some excellent symposia are not still being held. We try to conduct some good ones in this JOURNAL. The Ciba Foundation is justly famous for its already impressive catalog of symposia on all manner of medical subjects. And there are a few others supported by pharmaceutical manufacturers as well as by journals, medical societies, and philanthropic organizations that are very good indeed—but where to find them and how to know which will be worth while?

Certainly, we seem to be on a symposium "kick" in which much indiscriminate material is shoveled out under the special benediction it acquires by being part of the proceedings. The title alone gives the uncontrolled mutterings some stature. Then the statements made take on further importance because they all are published. Many symposia are mere repetitions of similar meetings held elsewhere with much the same cast of players. Indeed, it is often not difficult to predict who will hold forth on particular subjects. These performers would seem to proceed from one stopover to the next like the old Chautauqua circuit lecturers. What it can be I do not know, but there must be some sort of compensation for the weary travelers; in any event, it is clear that, since each performance assures them another publication in some sort of book, booklet, monograph, "proceedings," or brochure, these workers quickly build up a massive list of publications.

What is the point of a gathering to which the majority of speakers have been

invited to repeat what is already well known they will say. At best, it ensures a tired performance. So often that it cannot be a coincidence, all disputants monotonously express the same point of view. This seems to have been conveniently arranged in advance to prevent feather ruffling, unlike the old-fashioned symposia in which speakers were specifically chosen to invoke dispute as well as discussion. A symposium now also implies a sounding board for the "latest" on new developments which, in the Madison Avenue code, means premature and poorly digested statements, statements which would never pass the editorial referees of a first rate medical journal. It is a device to have unpublishable stuff published. Quality, bias, thoroughness appear to matter little. Enough stuff to fill a monograph seems to be the goal.

It has become less easy for me to be trapped by the program director who has very skillfully interwoven the interesting papers with filler so that there is no escape from listening to the junk. My recent opinions are based mainly on attendance forced on me when I found myself inveigled into playing a part in them and on my reading, which is voluntary and substantial. The latter is a far more satisfactory method of sampling than attendance because one can cover more symposia that way (and at home, glory be!). One does not miss a thing either, since, because of unanimous editorial purity on this one point, not a single word uttered at these meetings is ever mislaid. This I can attest to from personal experience. I am not a modest man but even I think they go too far. At one symposium at which I sat on a panel, my sole contribution to the entire proceedings was to respond to a question with "No." I cannot recall the question. Only my answer is now memorable. Not only was this gem published verbatim alongside my name but it apparently was a sufficient contribution to lead to my inclusion with a stretched-out account of my titles and connections in the list of *contributors* and to the use of my name in advertisements for the sale of the symposium in book form.

Why are so many symposia so devastatingly boring? Why is not their prime purpose the promulgation of knowledge? Why do they repeat themselves ad nauseum? Why are there so many cursorily collected laboratory and clinical observations reported? Why so much unfinished business? Why are they so full of insubstantial mental meanderings? In this age, when there is much that is both new and difficult, who can afford to waste time on the tripe that is served up at so many symposia? Why are no editorial scissors used before publications? In court records it is essential that every spoken word be taken down (and it is conceded that the verbatim records of even the most exciting murder cases are shatteringly dull), but why is this procedure used for scientific meetings?

The scientific editor must function as an editor, not as proofreader for a court stenographer. For me the editor's proper function starts early—in the programming. His objective should be to have a few good speakers and a lot of time for lively provocative discussion and thinking. All this seems so clear to me that it should also be to those involved in arranging symposia. Why has the symposium come to such a pass?

It is my opinion that this has happened because symposia are being used for something other than that for which they are eminently suited: the extension of knowledge. A rapidly increasing number of symposia are being held for the sole purpose of drug promotion. All expenses, travel allowances for invited speakers, costs of publication, honoraria, free distribution of the published volume (with royalties to the organizer-editor of the symposium) are frequently footed by pharmaceutical manufacturers. I have been informed quite candidly that this is the *cheapest* form of prestige obtainable for drug promotion. That is why there is no interest in quality; that is why premature and immature statements are encouraged; that is why they are

so one sided; that is why there is so much emphasis on publication and republication of the same drivel. And that is why they are such excruciating bores!

Why has this been permitted to happen to such a venerable educational instrument? The surprising truth is that substantial medical societies are often parties to these rigged performances. Even such an ivory-towerish institution as the New York Academy of Sciences is not above it; in fact it seems to have become a regular "scientific" feature. Surely the Council of the Academy realizes that to its list of superb publications some incredibly bad ones have been added as a result of this practice. And if such an institution does this, is it at all surprising that lesser organizations all over the country do likewise?

I have no quarrel with honest advertising of drugs—outright advertising. This is the American way, and that it is not inconsistent with the publication of scientific and unbiased information on medical matters is proved by the large number of excellent medical journals which accept advertising matter, which literally depend on it for their existence. It is this arrangement which makes it possible for this JOURNAL to operate and hope to continue to operate. But an advertisement should be called an advertisement. An advertisement in a medical journal may not be presented as if it were part of the text. In the same way, a collection of biased promotional speeches for a drug must not be presented as if it were an unbiased educational forum. I take issue with all who are parties to this perversion of an important postgraduate educational instrument into an insidious promotional device. There is the real danger that, should the symposium continue to be degraded by this practice, good symposia will disappear. Perhaps we need a Colloquium on the Symposium. Maybe Madison Avenue will give it back to the Indians.

Walter Modell

Commentary

Monoamine oxidase inhibition

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It cannot be denied that monoamine oxidase is an active and interesting substance, but there must be reservations about the biologic role and clinical importance of this enzyme. In particular, experimental findings do not seem adequate to support interpretations now being drawn as to modes of central actions of drugs, even though some of the postulates make a pretty picture.

The postulates start from the accepted fact that monoamine oxidase does react, at least in isolated systems, with 5-hydroxytryptamine (serotonin), dopamine, epinephrine, levarterenol (norepinephrine), and other catecholamines. These enzyme substrates are the substances presumed to be important in transmission at synapses in the central nervous system. By inactivating dopamine, a precursor of epinephrine and norepinephrine, monoamine oxidase might regulate synthesis of these amines in neural tissue. And by utilizing as substrates the catecholamines and serotonin, the enzyme might control or terminate central actions of these transmitters. There is evidence that these central actions are prominent. Serotonin and catecholamines are normally found in neural tissues, and it is theorized that serotonin is a mediator for excitatory impulses in the central nervous system while catecholamines serve as both inhibitory and excitatory transmitters. If monoamine oxidase metabolized these agents, drugs which inhibit the enzyme might be expected to alter central function. The hypothesis is supported by demonstrated inhibitory activity of monoamine oxidase of several centrally acting drugs. "Psychic energizers" are held to be especially active in this regard. Hydrazine derivatives of sympathomimetic amines, e.g., iproniazid (Marsilid) and phenylisopropyl hydrazine (Catron), are irreversible inhibitors of monoamine oxidase. Other hydrazine compounds unrelated to adrenergic agents, e.g., isocarboxazid (Maraplan), have the same characteristic.

Things have gotten to such a state that drugs are now introduced for clinical use primarily on the basis of demonstrated ability to inhibit monoamine oxidase, with implication that such activity will furnish beneficial central effects. It might be wise to recall that for a long time there have been attempts to find some physiologic function for monoamine oxidase. One of the older attempts was assignment to this enzyme of essential roles in peripheral metabolism of amines and explanation of peculiarities of ephedrine and amphetamine solely in terms of oxidase antagonism. Such theories have disintegrated with time for lack of real evidence that monoamine oxidase has any role in peripheral sympathetic function, and present certainties may well follow suit.

There is difficulty though in evaluating present concepts pertaining to central roles

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of this enzyme because so little meaningful work has been done. Investigators generally appear content to demonstrate some central activity of a drug, then to explain mechanisms in terms of monoamine oxidase inhibition as if this were an established fact. The point upon which more consideration is needed is not whether drugs do something but whether they really do it by inhibition of this specific enzyme.

Laboratory support for the theories can be criticized. Monoamine oxidase is tricky stuff with which to work; the material appears to vary from species to species and among tissues. In many studies, concentrations in urine of degradation products of epinephrine and norepinephrine have been taken to indicate degrees of inhibition of monoamine oxidase after administration of a presumed enzyme inhibitor, without proof and with disregard of other pathways of metabolism. Many in vitro demonstrations of enzyme inhibition by drugs have used concentrations of agents in excess of those obtainable from therapeutic doses. It is possible to block in vitro any enzyme if enough of anything is applied; it cannot be assumed that enzyme inhibition observed at one dosage will occur at a much lower one.

Clinical data must also be looked upon with reservation. There is insufficient attention paid to distinguishing effects of newer "psychic energizers" from those of the amphetamines. The latter increase psychomotor activity but do not improve orientation of the depressed patient. Since amphetamines do block monoamine oxidase and are as effective in this respect as other compounds, it would appear to follow that any additive effects, beyond those typical of amphetamines, could not be due to inhibition of the enzyme. In general there appears to be a complete lack of correlation between inhibitory potency and therapeutic effectiveness. Compounds which are quite active on the enzyme may be physiologically inert, and compounds which have no action on oxidase, such as imipramine (Tofranil), can alter psychic or emotional activity. Very few studies have made quantitative approaches to this problem. A few recent publications which have made the attempt may be cited, because in themselves they cast serious doubt upon the theories under consideration. Green and Erickson[1] have shown that there is no correlation in time between changes in brain concentrations of norepinephrine and inhibition of monoamine oxidase. An explanation for the discrepancies was afforded in the demonstration that another enzyme, orthomethyl transferase, acted upon the same substrates and at the same time as did monoamine oxidase and that this enzyme took over inactivation of catecholamines when oxidase was inhibited. There was nothing to point to any essential role for monoamine oxidase. Then Resnick and associates[2] studied relationships in man between enzyme inhibition in vivo and in vitro and central effects. It was found that no correlation existed in the parameters in the cases of isocarboxazid, iproniazid, isoniazid, and an experimental compound. A compound with high antienzyme potency would show no clinical effect, while another with no in vitro activity against the enzyme did change central function.

There does not appear to be proof that monoamine oxidase plays a critical role in regulation of transmission in the central nervous system, if it is involved at all. It appears certain that its inhibition is not *the* means by which centrally acting compounds produce effects. It remains to be demonstrated if oxidase inhibition is secondarily or indirectly involved in actions of drugs or if the enzyme-inhibiting activity of such agents is merely an unrelated coincidence. The latter seems likely.

References

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Effect of epinephrine, insulin, and tolbutamide on carbohydrate metabolism during ether anesthesia

The administration of epinephrine during ether anesthesia in man produced a greater than normal rise in blood glucose and elevations in pyruvate, lactate, and citrate levels less than would be expected from a combined effect of ether and epinephrine. Epinephrine failed to depress serum inorganic phosphorus levels or to prevent the progressive rise in levels observed during ether anesthesia. The administration of insulin during ether anesthesia failed to depress blood glucose and inorganic phosphorus levels. As with epinephrine, the changes in levels of pyruvate, lactate, and citrate were less than those expected from the combined effects of ether and insulin. Increased sensitivity to insulin was observed during thiopental anesthesia. The data suggest that ether may alter the cellular transfer and phosphorylation of glucose in a manner not fully explained by the reflex release of endogenous epinephrine.

The administration of tolbutamide during ether anesthesia depressed both blood glucose and serum inorganic phosphorus levels and prevented the progressive rise in glucose normally observed during ether anesthesia. The effect of tolbutamide administered during ether anesthesia suggests that it may have a glycostatic effect independent of its effect on insulin secretion.

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Alterations in glucose metabolism during ether anesthesia have long been recognized, but their significance has hitherto not been established. Previous studies in man[4,6,9,12] during anesthesia with ether, thiopental, or cyclopropane, under conditions of fasting or administration of glucose, have demonstrated alterations in peripheral blood concentrations of glucose and changes in certain carbohydrate me-

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Table I. *Effect of ether anesthesia on venous blood levels of carbohydrate metabolites and inorganic phosphorus in 31 patients**

Blood values (mg. per 100 ml.)	*Before anesthesia*	*Minutes of anesthesia*			
		30	*60*	*90*	*120*
Glucose					
Mean	73	101	125	126	128
S.E.	2.4	5.2	7.3	7.3	9.5
Pyruvate					
Mean	1.12	1.49	1.64	2.15	2.68
S.E.	0.09	0.13	0.15	0.26	0.37
Lactate					
Mean	8.1	14.5	16.5	21.2	20.0
S.E.	0.4	1.5	1.3	1.2	1.1
Citrate					
Mean	2.07	1.92	1.94	2.10	2.40
S.E.	0.17	0.18	0.203	0.33	0.33
Serum total inorganic phosphorus					
Mean	3.47	3.60	4.01	4.24	4.64
S.E.	0.06	0.10	0.16	0.16	0.17

*Of the 31 patients included in this series, 27 were studied at the Massachusetts General Hospital, Department of Anesthesia, with Dr. John P. Bunker.[3]

tabolites and inorganic phosphorus. Elevations in inorganic phosphorus were found to be common to all three agents with varying degrees of intolerance to administered glucose intravenously. Rises in the levels of organic acids (lactate, pyruvate, citrate, and alpha-ketoglutarate) were observed during cyclopropane or diethyl ether anesthesia, while a decrease was found during thiopental anesthesia.*[9] Concentrations of ketones and free fatty acids also increased during ether anesthesia.*[9]

While some of the changes during ether anesthesia could be explained in part by endogenous release of epinephrine[2] or a possible decrease in renal phosphorus excretion, these mechanisms were not deemed responsible for the changes observed during thiopental anesthesia. Similarly, while alterations in respiratory acid-base balance might explain the observed changes in organic acid levels during thiopental anesthesia, they were not applicable to the changes observed during ether or cyclopropane anesthesia.*[3] In addition, the persistent increase in serum inorganic phosphorus, after fasting or when glucose was administered during anesthesia with all agents, could not be explained by any of these mechanisms. In view of these observations it was postulated that anesthesia may in some manner alter the mechanisms whereby the cellular transfer and phosphorylation of glucose are accomplished.[12]

The present report concerns further investigations of the changes in carbohydrate metabolism produced by ether anesthesia. Effects of epinephrine, insulin, and tolbutamide administered during anesthesia were examined in an effort to define more precisely the manner whereby ether might interfere with cellular transfer of glucose.

*D. H. Henneman and J. P. Bunker: Data in preparation for publication.

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Table II. *Effect of epinephrine administered to 6 nonanesthetized subjects and to 12 subjects anesthetized with ether*

Blood values (mg. per 100 ml.)	*Nonanesthetized subjects*					*Anesthetized patients*				
	Minutes after epinephrine					*Before ether*	*Minutes after epinephrine*			
	0	*30*	*60*	*90*	*120*		*0**	*30*	*60*	*90*
Glucose										
Mean	82	132	160	132	118	72	154	207	251	219
S.E.	3.5	12.2	16.8	5.4	6.2	7.8	27.4	16.6	29.0	33.2
Pyruvate										
Mean	1.3	1.82	2.20	1.75	1.4	1.3	1.6	2.07	2.55	2.65
S.E.	0.17	0.22	0.20	0.50	0.4	0.3	0.10	0.22	0.40	0.30
Lactate										
Mean	7.5	18.5	20.0	18.5		6.0	12.8	18.5	23.6	29.1
S.E.	0.8	1.3	2.1	0.9		0.8	0.8	2.9	3.6	4.5
Citrate										
Mean	2.1	2.5	2.9	2.7	1.8	1.77	1.57	2.87	2.27	1.82
S.E.	0.2	0.3	0.3	0.2	0.2	0.41	0.34	0.58	0.39	0.29
Inorganic phosphorus										
Mean	3.65	3.10	2.95	3.1		3.7	3.86	3.93	4.50	5.01
S.E.	0.10	0.10	0.30	0.1		0.17	0.15	0.17	0.18	0.52

*This sample was taken 1 hour after induction of ether anesthesia.

Case material and methods

Apparently healthy men and women under 50 years of age who were to undergo relatively uncomplicated surgical procedures* were prepared with a high carbohydrate diet and an overnight fast prior to the day of operation.[10,14] Preanesthetic medication consisted of 100 to 200 mg. pentobarbital by mouth and 0.4 to 0.6 mg. atropine sulfate by hypodermic injection. Anesthesia was induced with 50 to 100 mg. thiopental intravenously followed by nitrous oxide in a semiclosed and ether in a closed circle carbon dioxide absorption system. Respirations were spontaneous throughout after tracheal intubation with the aid of a 40 mg. dose of succinylcholine. The major share of the metabolic studies was performed 30 to 60 minutes after induction of ether anesthesia, in the second and third planes of the surgical stage, and usually before the commencement of surgical operation. A slow, intravenous infusion of 0.8 per cent saline (100 to 200 ml. over a period of 2 to 3 hours) was administered; no other fluids were given. Epinephrine in a subcutaneous dose of 0.01 mg. per kilogram of body weight or intravenous tolbutamide in a dose of 1 Gm. was given 30 to 60 minutes after induction of anesthesia. *Unanesthetized,* normal adult men and women were studied after similar doses of epinephrine, insulin, or tolbutamide: 12 subjects received epinephrine,* 8 received insulin, and 8 tolbutamide. Peripheral venous blood samples were collected in all subjects. Analytic techniques were the same as those previously described[10]: glucose—Nelson and Somogyi, J. Biol. Chem. 153:375, 1944; pyruvate—Selig-

*Surgical procedures included excision of thyroid nodules in euthyroid individuals, breast biopsy, herniorrhaphy or cholecystectomy, dilation and curretage, and hysterectomy.

*The group of individuals who received epinephrine included patients in the series studied earlier by Hennemen, D. H., and Altschule, M. D.: A.M.A. Arch Int. Med. 95:594, 1955.

son and Shapiro, Anal. Chem. 24:754, 1952; lactic acid—Barker and Summerson, J. Biol. Chem. 138:535, 1941; citric acid—Elliott modification of Natelson and colleagues, J. Biol. Chem. 175:745, 1948; and inorganic phosphorus—Fiske and SubbaRow, J. Biol. Chem. 66:375, 1925.

Results

Epinephrine administration. The administration of epinephrine during ether anesthesia in 12 patients increased blood glucose concentrations to a mean level of 219 mg. per 100 ml. after 90 minutes, which was higher than that observed in the unanesthetized subject (132 mg.) (Table II). The rise of 147 mg. was greater than that expected from a combined effect of ether (53 mg.) and epinephrine (50 mg.). Concentrations of pyruvate and lactate generally increased during ether anesthesia alone; when epinephrine was injected, no remarkable change was observed (Tables I and II).

Insulin administration. The intravenous injection of insulin during ether anesthesia was associated with a variable metabolic response which appeared dependent upon the depth and duration of anesthesia both prior to and following the injection of insulin.

1. Effect of insulin administered intravenously during the second and third planes of surgical anesthesia. Blood glucose levels failed to decrease in the expected manner when insulin was injected intravenously during anesthesia maintained at anesthetic planes 2 to 3 for 2 hours after the injection (patients 7 to 12, Table III). A mean maximal decrease of 39 mg. per 100 ml. (a 23 per cent fall from the level immediately prior to insulin) occurred 45 to 60 minutes after insulin injection. This decrease was significantly less ($p < 0.01$)

Table III. *Effect of insulin on blood glucose (mg. per 100 ml.) during ether anesthesia in 12 patients and in 8 normal, nonanesthetized subjects*

Patient numbers	*Before ether*	*Minutes of ether*	*After ether*	*After insulin—during ether*					
				Minutes after insulin					
				20	*30*	*45*	*60*	*90*	*120*
1	61	30	133	90 L	48 L	32 L	45 L		98 A
2	73	60	104		62 L		57 A		65 A
3	60	20	78 L		56	43	63 L		67 A
4	52	40	84 L	60			46	57 L	63 A
5*	130	60	238	193	132		108 L		80 A
6	75	60	130	80 L	55 A		55 A		
7–12									
Mean	89	60	171	155	140		133	140	130
S.E.	7		14	15	13		13	9	9

Normal subjects (8)	*Before insulin*	*After insulin—without ether*					
		20	*30*	*45*	*60*	*90*	*120*
Mean	81	47	40	49	59	74	75
S.E.	6	15	12	11	7	4	7

L = light anesthesia; A = awake and responsive; all other values were obtained during surgical anesthesia (planes 2 to 3).

Surgery was begun 1 hour after the administration of insulin except with patients 1, 2, and 8.

Anesthesia was monitored by electroencephalographic, electrocardiographic, and blood pressure tracings; no significant changes occurred after the administration of insulin.

*Patient 5 had a stormy period of induction with respiratory distress, coughing, and muscular activity; note how this increased the sensitivity to administered insulin.

and considerably more delayed in onset than that observed in a series of 8 normal subjects who received insulin in the absence of anesthesia (Table III) and from that reported in the literature. Insulin did not produce an increase in levels of blood pyruvate and lactate in excess of that expected from ether alone; the changes observed were less than those expected from a combined effect of ether and insulin (Tables I and IV). Insulin, under these same anesthetic conditions, also failed to depress citrate and serum inorganic phosphorus in the normal fashion[18] or to prevent the rise in these constituents observed during anesthesia alone (Tables I, IV, and V).

2. *Effect of insulin administered during light ether anesthesia or anesthesia of short duration; comparison of effects noted during thiopental anesthesia.* Blood glucose not only decreased significantly below the preinsulin level but also below the preanesthetic concentrations when insulin was

Table IV. *Effect of insulin on blood pyruvate, lactate, and citrate levels during ether anesthesia*

Metabolites	*Venous blood concentrations (mg. per 100 ml.)*						
	Before ether	*After 30 to 60 minutes of ether*	*Minutes after insulin*				
			20	*30*	*45*	*60*	*120*
Pyruvate							
Mean	1.3	1.5	1.8	2.0	1.9	2.3	2.2
S.E.	0.3	0.3	0.3	0.3	0.6	0.5	0.4
Lactate							
Mean	5.6	10.5	14.8	17.1	18.0	17.7	17.7
S.E	0.1	1.7	2.1	2.4	2.8	2.7	2.8
Citrate							
Mean	1.37	1.33	1.53	1.46	1.73	1.9	2.0
S.E.	0.17	0.18	0.20	0.21	0.20	0.18	0.33

Table V. *Effect of insulin on serum total inorganic phosphorus during ether anesthesia in 12 patients*

Patient numbers	*Before ether*	*Minutes of ether*	*After ether*	*After insulin—during ether*				
				Minutes after insulin				
				20	*30*	*45*	*60*	*120*
1	2.1	30	2.3	2.8	3.0 L	3.3 L	3.6 L	3.1 A
2	3.3	60	3.1 L	3.1 L	3.0 L	3.1 L	3.6 L	3.7 A
3	2.7	60	3.4		2.7	3.0 L	3.2 A	3.5 A
4	3.0	20	3.2		2.7	2.2	3.1 L	3.5 A
5*	2.4	40	2.6			3.4	3.3 L	3.3 A
6	3.7	60	4.5	5.0	4.3 L	4.4 A	4.4 A	2.5 A
7–12								
Mean	3.0	60	3.6	3.6	3.7	3.9	4.0	4.1
S.E.	0.2		0.12	0.01	0.13	0.10	0.16	

L = light anesthesia; A = awake and responsive; all other values were obtained during surgical anesthesia (planes 2 to 3).

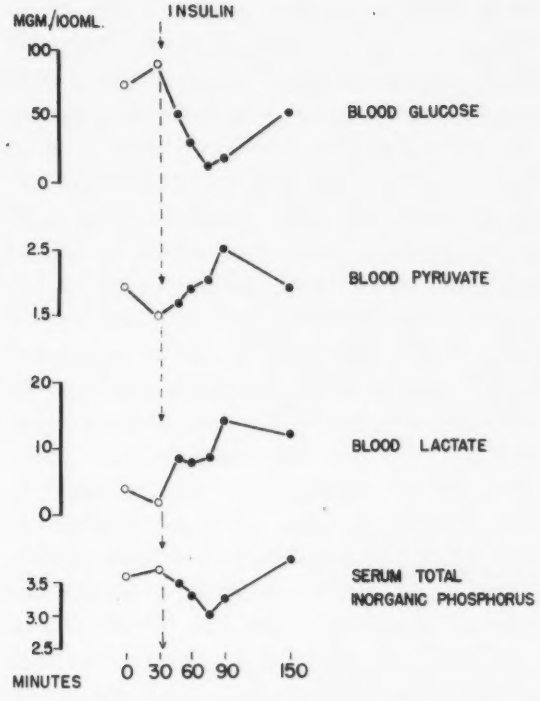

Fig. 1. Effects of insulin administered during thiopental anesthesia in a normal adult male.

injected after less than an hour of anesthesia or when anesthesia was discontinued within 2 hours of the injection of insulin (patients 1 to 6, Table III). Under these anesthetic conditions, changes in serum inorganic phosphorus levels were variable. Patients 1 and 2 (Table V) still failed to show any significant decrease, while patients 3, 4, and 6 showed a significant fall after insulin.

Insulin was administered to a normal adult male during the course of thiopental anesthesia for an inguinal herniorrhaphy. In contrast to the changes observed during ether anesthesia (planes 2 to 3 of the surgical stage), blood glucose levels fell to 13 mg. per 100 ml. within 45 minutes and remained below 20 mg. per 100 ml. for as long as 90 minutes after insulin (Fig. 1). The degree and duration of hypoglycemia observed were greater than normal[7] and in excess of that observed in this same patient when not anesthetized. The changes in pyruvate, lactate, and citrate induced by insulin during thiopental anesthesia were comparable to those observed in the nonanesthetized state. These findings do not answer the question whether the alterations during ether anesthesia were the result of a greater depth of narcosis or a qualitatively different metabolic effect.

3. Effects of tolbutamide administration during planes 2 to 3 of the surgical stage of ether anesthesia. Tolbutamide administered intravenously to 8 unanesthetized, normal subjects decreased levels of blood glucose, pyruvate, lactate, and serum inorganic phosphorus in the expected manner.[13] Serum citric acid also decreased (Fig. 2). When administered to 8 patients

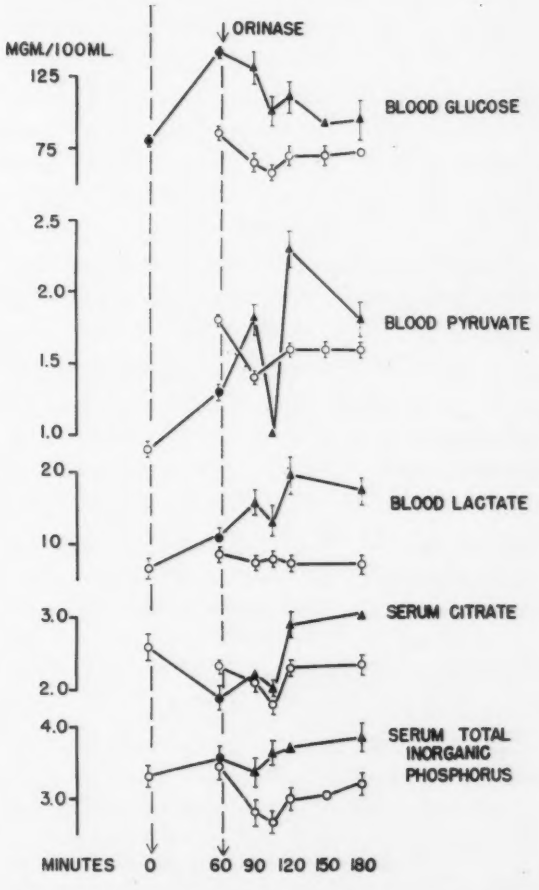

Fig. 2. Effect of tolbutamide (Orinase, The Upjohn Company) administered during ether anesthesia. Mean changes in 8 anesthetized subjects who received 1 Gm. tolbutamide intravenously (solid triangles) are compared to the mean changes in 8 nonanesthetized subjects who received tolbutamide (open circles). Standard error of the mean is indicated by the symbol *I*.

during ether anesthesia, tolbutamide not only decreased blood glucose levels but prevented the progressive and prolonged rise of glucose normally observed during anesthesia (Fig. 2 and Table I). Although tolbutamide produced an initial fall in levels of pyruvate, lactate, citrate, and serum inorganic phosphorus, it failed to prevent the subsequent, progressive rise observed during ether anesthesia.

Discussion

Previous work[2] has suggested that the simultaneous rise in venous blood levels of glucose, pyruvate, and lactate observed during ether anesthesia in the dog was a reflection of increases in the endogenous release of epinephrine since these metabolic changes were prevented by total sympathectomy. However, the metabolic acidosis observed in the dog was far greater than that found in man during ether anesthesia,[3] and Price and associates[19] have shown that norepinephrine rather than epinephrine is the major sympathetic hormone secreted in man. Thus, a species difference has been demonstrated and suggests that the change in carbohydrate metabolism noted in man may be the result of some other mechanism. The results of the present study in man, however, indicate that curtailment of insulin action in deep anesthesia will of necessity exaggerate the hyperglycemic response to exogenous administration or endogenous secretion of epinephrine.

Ether anesthesia in man is associated with elevation in the level of serum inorganic phosphorus after both preanesthetic fasting and the administration of glucose.*[4,6,9] A definite degree of glucose intolerance has also been found.[4,9] The present data extend these observations and indicate that ether anesthesia is also associated with insulin resistance. Both insulin and epinephrine failed to depress inorganic phosphorus levels, and epinephrine caused excessive rises in blood glucose without the expected increases in pyruvate, lactate, and citrate. These findings suggest that ether may not only increase hepatic glycogenolysis but may also interfere with cellular transfer of glucose thereby impeding the phosphorylation and subsequent metabolism of glucose. If this interpretation is correct the rise in blood glucose may be assumed to be the result of an alteration in the distributional space for glucose. Although epinephrine per se has been shown to decrease the uptake of glucose by tissues[5] and perhaps also to alter glucose phosphorylation,[17] epinephrine administered to nonanesthetized man will lower serum inorganic phosphorus levels. The latter effect is presumed due to endogenous insulin secretion secondary to the rise in blood glucose levels. However, as the present studies indicate, the decrease in phosphorus does not occur when epinephrine is administered during ether anesthesia. Hence it must be postulated that factors other than the endogenous release of epinephrine are responsible for the abnormal elevation in serum inorganic phosphorus observed during ether anesthesia in man.

Other than interference with glucose phosphorlyation and cellular transfer, it has been suggested that ether inhibits certain specific metabolic pathways. One of these is the in vitro uncoupling of oxidative phosphorylation,[2] a phenomenon observed with many other drugs.[16] A selective effect of ether on glucose metabolism was indicated by the studies of Drucker[4] wherein little alteration in the assimilation of fructose was observed under the same conditions of anesthesia. These data do not rule out, however, the postulated effect of ether on the cellular transfer of glucose.

A decrease in pyruvate and lactate after the adminstration of tolbutamide in nonanesthetized man has been reported by others.[13] A simultaneous decrease in serum citrate levels was observed in the present report. Sulfonylthiourea derivatives have been reported to depress pyruvate and lactate levels in dogs anesthetized with bar-

*D. H. Henneman and J. P. Bunker: Data in preparation for publication.

biturates.[8] In man, thiopental anesthesia alone will decrease levels of pyruvate, lactate, citrate, and alpha-ketoglutarate*; comparable stuides have not been done in dogs. We have not observed prolonged decreases in pyruvate and lactate levels when tolbutamide was administered to man anesthetized with ether.

The fact that tolbutamide maintained a depression of blood glucose during ether anesthesia while insulin did not suggests that the former compound has a glycostatic effect as well as an effect on insulin secretion.[8] The persistence of elevations in lactate, pyruvate, and citrate during ether despite the depression in blood glucose after tolbutamide is not understood, in view of the decreased amount of glucose available to the tissues. However, blood glucose levels per se are not responsible for the effects of glucagon on the metabolism of pyruvate, lactate, or citrate,[11] and a comparable situation may exist during ether anesthesia and/or the reflex release of endogenous epinephrine.

Insulin resistance was not found during thiopental anesthesia in the 1 patient studied. The abnormally prolonged depression of glucose levels suggests a decreased hepatic glycogenolytic response to hypoglycemia and is consistent with the recent report that thiopental depresses adrenal medullary secretion of epinephrine and norepinephrine.[20]

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I will now bring this "perspective" to a close by putting in a plug for the middle estate in medicine, where, after all, my life has been spent.

The middle estate in medicine is where the three functions of medicine—research, teaching, and practice—are most sweetly blended. And if, to be sure, it is those who follow exclusively a single scientific beam who make the most signal advances, it is also true that their contributions may long lie fallow unless there is development by those whose field of competence is described by a wider angle. Consider penicillin. After Fleming discovered it, nothing further happened for thirteen years until Florey proved its medical value and put it into clinical use and A. N. Richards got it into mass production, thus revolutionizing medicine throughout the world.

The full-time clinicians make and maintain teaching clinics which are the germinative centers both of new knowledge and of the people who create the forward march of medicine. None but those who love medicine and strive for excellence should go into this field.

Studies of analgesic drugs

V. The comparative subjective effects of oxymorphone and morphine

Oxymorphone, a derivative of morphine, is a potent, addicting analgesic capable of producing both respiratory and circulatory depression. It has achieved wide use because of the low incidence of undesirable gastrointestintal actions reported in extensive clinical trials with patients having pain of cancer.

This study was undertaken to quantitate the relative subjective side action liability of oxymorphone and morphine in patients who were free of pain. Equivalent analgesic doses of morphine (10 mg. per 70 kg.) and oxymorphone (1.05 mg. per 70 kg.) were given to two groups of hospitalized patients who were awaiting elective surgical operations. Nausea and vomiting were significantly more frequent and severe after oxymorphone than after morphine. At this dose, oxymorphone produced sedation, dizziness, and other typical morphinelike effects as frequently as did morphine. The time action curve of oxymorphone was similar to that of morphine when expressed in terms of subjective effects. Reasons for the discrepancy between these results and those of clinical trial are discussed.

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Oxymorphone (14-hydroxydihydromorphinone),* a recently produced morphine derivative,[15] has proved to be a potent analgesic both in animals and man.[2,3,6] Although it possesses high addiction liability[7] and is capable of producing hypotension and respiratory depression, oxymorphone has continued to arouse interest because of its reported low incidence of undesirable gastrointestinal actions. Coblentz and Bierman,[3] in reporting their experience with more than 9,000 doses of oxymorphone in 200 patients, stated they observed no nausea, vomiting, or constipation with doses up to 10 mg. Samuels and associates[11] reported nausea or vomiting in only 3 out of 45 patients with pain of cancer treated chronically with oxymorphone at doses up to 5 mg. Eddy and Lee[5] observed only one instance of nausea and no vomiting after 167 doses of oxymorphone of 1.33 mg. or less. With doses of 1.5 and 2 mg., nausea and vomiting occurred with approximately the same frequency as followed 12 mg. of morphine. In all three studies, observations on the frequency of undesirable effects were made incidental to the treatment of pain

This study was supported by a grant from Endo Laboratories, Inc., Richmond Hill, N. Y., who also provided the oxymorphone used in this study.
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*Numorphan.

in patients with neoplastic diseases. Such observations have been found by us and others[1] to be difficult to interpret quantitatively, since it is often impossible to distinguish undesirable effects of drugs from the manifestations of the disease being treated. The following study was therefore undertaken to compare quantitatively the relative subjective side action liability of oxymorphone and morphine.

Methods

Two groups of female patients who were awaiting elective surgical operation, most commonly gynecologic, were the subjects of this study. Only female patients were used, to provide more homogenous samples. Patients were considered suitable for study if they were not seriously ill and were not receiving other medications. On the afternoon before operation, which was usually the day of admission to the hospital, patients were given either morphine (10 mg. per 70 kg.) or oxymorphone (1.05 mg. per 70 kg.) intramuscularly by the ward nurse without explanation as to the purpose of the injection. A technician who did not know the drugs under study assigned alternate patients to one of the two drugs until 30 patients were obtained in each group. No patient received more than one drug. All drugs were coded and the code was changed in the middle of the study. All patients were interviewed by one technician before and at 30, 60, and 120 minutes after every injection. Three types of data were collected:

A. Subjective. The presence or the absence of a variety of symptoms was recorded, for example, dizziness, sleepiness, nervousness, etc. A list of signs and symptoms of special interest was used by the technician as a guide for recording drug effects. In addition, verbatim statements of the patients were recorded when pertinent. Information was obtained only in response to nonspecific questions such as "How do you feel?"

B. Objective. Signs such as restlessness, perspiration, and vomiting were noted.

C. Value judgments. The technician estimated the following characteristics: more or less cheerful, sedated or stimulated, pleasant or unpleasant drug effect, and degree of drug effect (little, moderate, or great).

To estimate intensity of the subjective effects as well as incidence, a scoring system was used. At each observation period the reported signs and symptoms were graded in severity as follows: 10—slight, 20—moderate, or 30—marked. For each effect observed, a total 2 hour score was calculated for each patient. The mean of the 30 and 60 minute scores was added to the 120 minute score to obtain a 2 hour effect score. These scores provided the basis for comparisons of total drug effects and for

Table I. *Percentage incidence of subjective effects in two groups of 30 female patients after morphine and oxymorphone*

Data	*Morphine (10 mg. per 70 kg.)*	*Oxymorphone (1.05 mg. per 70 kg.)*
White	17	23
Over 40 years	37	33
Drug effect		
Sleepiness	69*	90*
Drunk feeling	20	13
Grogginess	13	27
Nervousness	7*	27*
Relief of nervousness	3	3
Dizziness	73*	100*
Sight difficulty	30	43
Heavy feeling	13	10
Perspiration	20	27
Hot feeling	30	43
Nausea	30*	57*
Vomiting	3*	23*
Itch	7	0
Dry mouth	10	3
Dislike of drug effect	63	73
Technician evaluation		
Cheerful	0	3
Less cheerful	13	17
Sedated	73	90
Little drug effect	30	23
Unpleasant drug effect	40	47

*Significant differences in percentage incidence at $P<0.05$.[18]

construction of time-effect curves. In some categories (sleepy, nervous, cheerful, and sedated) patients could report either an increase (positive) or a decrease (negative) effect. In calculating the 2 hour score, these signs were not ignored.

Oxymorphone hydrochloride at 1.05 mg. per 70 kg. was considered to be the dose producing analgesia equivalent to 10 mg. per 70 kg. of morphine sulfate, both considered as weight of the respective salts. This dose of oxymorphone was derived from the data of Wallenstein and Houde[14] and the data of Eddy and Lee,[5] who estimated, respectively, that 1.12 mg. and 1.02 mg. of oxymorphone was the analgesic equivalent of 10 mg. of morphine.

Results

The incidence of the most prominent subjective effects after morphine and oxymorphone is presented in Table I. The occurrence of any sign or symptom at one or more of the three observation periods after drug administration contributed only once to the group incidence. Except for the effects drunk feeling, heavy feeling, itching, and dry mouth, the frequency of all subjective effects was higher after oxymorphone than after morphine. These differences were statistically significant only for the traits sleepiness, nervousness, dizziness, nausea, and vomiting. Relief of nervousness was difficult to interpret, since subjects were not asked if they were nervous before injection. Only volunteered information was recorded. Sight difficulty included double vision, difficulty in focusing the eyes, and extreme dizziness. A heavy feeling usually referred to the extremities but at times to the head or "all over." A hot feeling included most but not all the patients who perspired; some patients who perspired profusely did not complain of feeling hot.

The results were similar when the 2 hour scores were used to compare subjective effects (Table II). Except for the characteristics drunk feeling and dry mouth, the mean score per patient for all effects was greater after oxymorphone than after morphine. However, these differences were statistically significant only for nausea and vomiting.

Table II. *Mean 2 hour effect scores per patient (± standard error of mean) for subjective effects after morphine and oxymorphone in two groups of 30 patients*

Drug effect	*Morphine (10 mg. per 70 kg.)*	*Oxymorphone (1.05 mg. per 70 kg.)*
Drunk feeling	3.5 ± 1.6	3.3 ± 1.5
Grogginess	2.7 ± 1.3	5.3 ± 1.6
Sleepiness	16.2 ± 2.7	22.3 ± 2.1
Nervousness	0.1 ± 1.7	4.2 ± 2.1
Dizziness	21.2 ± 2.8	25.2 ± 1.7
Cheerfulness	− 4.7 ± 2.3	− 5.7 ± 2.6
Perspiration	2.7 ± 1.4	5.5 ± 2.0
Feeling hot	5.3 ± 1.9	8.5 ± 2.2
Nausea	4.7 ± 1.6*	12.3 ± 2.6*
Vomiting	0.3 ± 0.3*	5.0 ± 2.0*
Sight difficulty	5.3 ± 1.6	7.7 ± 1.8
Dry mouth	1.3 ± 0.8	0.7 ± 0.7
Heavy feeling	1.7 ± 1.0	1.7 ± 1.0

*Means are significantly different at $P<0.05$.[12]

Since a more sustained peak action by oxymorphone could account for higher total scores, the time-action curves of the most prominent effects were plotted. Curves (mean score per patient at each observation period) were constructed for the traits sleepiness, dizziness, nausea, vomiting, hot feeling, perspiration, difficult focusing of eyes, and nervousness. Two curves are presented (Figs. 1 and 2). For all symptoms the time-action curves of the two drugs were similar. The peak effect always occurred at either the 30 or 60 minute observation period.

Discussion

The large discrepancy between the incidence of nausea and vomiting after oxymorphone in this study and the lack of nausea and vomiting reported in clinical trials merits comment. Several reasons can be postulated to explain this difference.

First, in reported clinical studies, observations on undesirable actions were made incidental to the treatment of pain from neoplastic diseases. It can be safely assumed

that many of these patients were bedridden. Ambulation is known to increase the incidence of nausea and vomiting after morphine[4] and probably this is true of other narcotics. It is also probable that many patients received some narcotic prior to oxymorphone administration and were therefore in some degree tolerant to narcotic effects. In contrast, the patients in this study were nontolerant and permitted to ambulate, both of which tended to increase nausea and vomiting.

Second, estimates of side action liability in patients who received narcotics for pain must be interpreted with caution, since the same medication which relieves the most urgent symptom of pain also alters the attitude toward less dominant symptoms such as dizziness and nausea. Such patients, relieved of pain, are not likely to report undesirable actions, in contrast to the symptom-free patients studied.

Finally, the patients in this study were in a nontherapeutic situation comparable to use of normal subjects for the estimation of side action liability. The circumstances of our study constituted an anxiety laden situation, since hospital admission and anticipation of operation provoke anxiety almost universally. These symptomless patients were not reluctant to volunteer information on the effects of a drug which significantly disrupted their well-being. The use of patients in such a stress situation for this purpose was designed to elicit side actions and to magnify differences between drugs, similar to the use of a carbon dioxide stimulus to magnify the respiratory depression of potent analgesics. Admittedly, the absolute incidence of undesirable actions will be greater in such subjects than when the same drug is used in a therapeutic situation. However, comparative data between drugs were being sought.

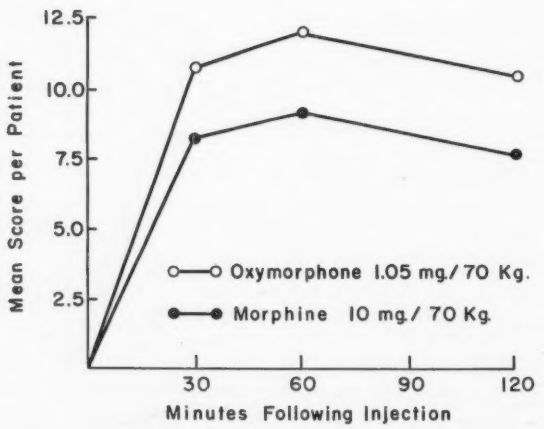

Fig. 1. Time-action curves for the trait sleepy expressed as mean score per patient at the three observation periods after injection.

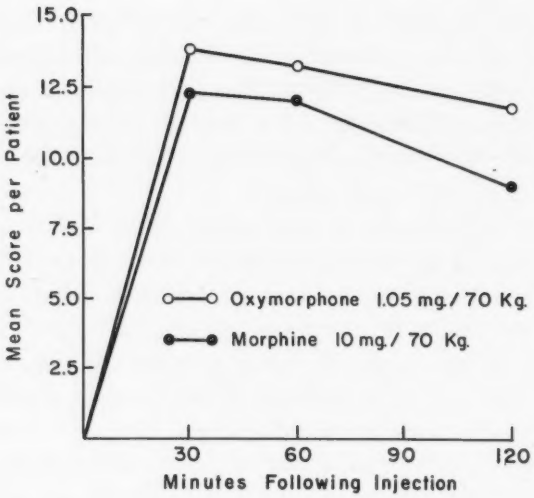

Fig. 2. Time-action curves for the characteristic dizzy after oxymorphone and morphine. The effect of both drugs is sustained 2 hours after injection.

The incidence of subjective effects after morphine reported here was not very different from that reported by us previously on similar patients.[8-10] Groups of 30 such patients have given consistent and reproducible results when used for estimating relative side action liability. Since a crossover study could not be done, it was possible, although unlikely, that the differences between morphine and oxymorphone noted here were the result of a sampling error. Even so, it was evident that oxymorphone did not possess lesser subjective side action liability than morphine and the claim of lesser gastrointestinal side actions after oxymorphone was not substantiated in this study.

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Influence of emylcamate, meprobamate, and placebo on psychologic test performance

With a double blind technique, emylcamate was compared with placebo and with meprobamate, a closely related compound, by a battery of psychologic tests which included sixteen measurable variables. In single doses of 1,200 mg., meprobamate depressed performance where emylcamate did not; in doses of 1,800 mg., both drugs depressed performance. These comparisons do not, however, distinguish any difference in effect on anxiety.

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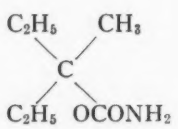
Searching for an agent that would have both potent tranquilizing and muscle relaxant properties, Melander[7] screened a large number of compounds that possess the minimal structural requirements thought to be necessary for such activities. From these compounds, 1-methyl-1-ethyl-propyl carbamate was selected for further studies and was given the generic name emylcamate.*



Emylcamate

In studies conducted by Melander, the properties of emylcamate were evaluated and compared to those of the related compound, meprobamate (2-methyl-2-n-propyl-1,3-propanediol dicarbamate).*

According to Melander, emylcamate proved to be very active in antagonizing convulsions induced by various chemical agents and electroshock in laboratory animals. Moreover, emylcamate appeared to produce relatively profound tranquilization at dose levels which did not affect skeletal muscles. It has low toxicity. In contrast to meprobamate, emylcamate has very rapid onset of action; after the administration of a single dose, the effect becomes maximal in several minutes, in about one-tenth of the time that is necessary for meprobamate to reach peak activity under identical conditions. In these experiments, milligram for milligram, emylcamate appeared to be about twice as potent as meprobamate.

Experiments have indicated that emylcamate is a highly active interneuron-block-

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*Nuncital, A/B Kabi; Striatran, Merck Sharp & Dohme, Inc.

*Merck Sharp & Dohme, Inc., Research Laboratories: Personal communication.

ing agent. In spinal cats, the drug inhibited reflexes mediated by polysynaptic neuronal pathways. Electroencephalograms in cats revealed similar slowing of spontaneous activity, without apparent changes in the specific sensory pathway conduction, after both emylcamate and meprobamate administration. Emylcamate only modified alerting responses and, even at high doses, rarely abolished the activation patterns. In monkeys with implanted electrodes, emylcamate and meprobamate rarely impaired and did not abolish the alerting responses to sensory stimuli at doses above those which reduced the frequency of spontaneous electrical activity. These results indicated that emylcamate, in doses that will induce an ataractic effect, is unlikely to produce sedation in man.

Methods

Scope of study. The results of laboratory experiments showing the increased activity of emylcamate compared with meprobamate were substantiated in clinical studies. Emylcamate, 200 mg. three times daily, produced tranquilization and relaxation comparable to that obtained with 400 mg. meprobamate given three times daily. Mårtens,[6] in a double blind study, found that emylcamate in amounts of 400 mg. three times daily produced significantly better responses in severe alcoholics suffering from neurosis than the same doses of either meprobamate or a placebo.

In preliminary studies, results of which are discussed below, meprobamate was shown in various tests to definitely impair performance at a dose level of 1,200 mg. It was, therefore, decided to use this dosage in subsequent tests for both emylcamate and meprobamate, despite the greater activity of emylcamate.

The investigation was designed as a double blind study. Identical, sugar-coated, 200 mg. tablets of emylcamate, meprobamate, or a placebo were used. In the first part of the study, 1,200 mg. of one of the three compounds was administered about 2¼ hours before the start of the tests and at least 2 hours after a light meal. (Melander[7] has shown in experiments on paralyzing activity in rabbits that the onset of emylcamate effect is very rapid, in a few minutes, and that the duration of activity is approximately the same for emylcamate and meprobamate.) Each subject received emylcamate and meprobamate once and placebo twice. At the beginning of these studies, it was decided that if a definite change from control was not obtained at the 1,200 mg. dose level, the dose would be increased by 50 per cent, i.e., to 1,800 mg., and the test repeated. It should be noted that the 1,200 mg. dose, which was given in a single administration, represents the total suggested therapeutic dosage of emylcamate for 24 to 48 hours and that of meprobamate for 24 hours.

Selection of subjects. In clinical studies by Kelly and associates[1] and by Kornetsky[3] and in some experiments of Reitan,[8] normal volunteers were used; Marquis and colleagues[5] also employed patients with neurosis and psychosis. Since patients suffering from anxiety and tension neuroses may react differently than "normal" subjects to these drugs, such patients were selected for these studies. The patients were almost ready for discharge from the hospital. The group that received 1,200 mg. doses of the drugs was comprised of 8 men from 40 to 59 years old (average, 48).

Testing procedures. The drugs were administered on successive days, at the same hour of the day each time, with one drug-free day on alternate days (two days at weekends). The patients received no other drugs during the test week. Performance was measured in a battery of tests which took approximately 2 hours.

The tests were assigned according to a modified Latin square design.

Psychologic tests

Simple reaction time and disjunctive reaction time. The procedures employed for measuring simple and disjunctive reaction time and those used in tapping experiments were essentially the same as the ones de-

scribed by King.*[2] The stimulus was supplied by a buzzer or bell.

The lift time is defined as the mean time lapse between the onset of the stimulus and the simple motor response of the subject, i.e., lifting the finger from the starting point (King[2]). Ballistic time is the mean time lapse between lifting the finger from the starting point and touching a metal plate placed at a distance of 26.5 cm. (Landis and Clausen[4]). Total time is the sum of lift plus ballistic time.

Tapping test. The mean number of finger taps made by alternately touching, during a 5 second period, two metal plates which are separated by a distance of 30.3 cm. is recorded.

Spoke's test.

Part A. The apparatus employed for Spoke's test was described by Reitan.[9] Twenty circles are arranged in a circular pattern. The starting point is at the center of the pattern, which has a diameter of 18 cm. In each circle is inscribed a number from 1 to 20, arranged randomly. At the signal the subject lifts his finger from the starting position and places it on circle 1, then on circle 2, and so on, until circle 20 is reached, returning the finger to the starting point between each number.

Part B. In this test, each of the twenty circles contains either a number (from 1 to 10) or a letter (the first ten of the alphabet) distribtued in a random manner. The subject has to pick alternately a number or a letter, taking care to preserve the correct numerical or alphabetical order. Reitan's test subjects proceeded from 1 to A, then 2 to B, etc., while in our experiments the subjects proceeded from 1 to 2, then from A to B, followed by 3 to 4, C to D, etc., returning to the starting position after each number or letter.

In both Part A and Part B of this experiment, each subject was given one trial and two regular test runs. Six different test patterns were employed both for Part A and Part B of the experiment. The score consists of the time required to complete the tasks, with all errors noted.

Vigilance. This test is a slight modification of a test by Wilkinson.[9] The subject is seated at a distance of 1 M. from a glass screen 20 cm. in diameter on which a small spot of light, only slightly brighter than the illuminated screen, occasionally appears. The spot of light may appear, for half a second, in any of eight equidistant positions on an imaginary circle. (The suitable light intensity of the small spots is determined for each test subject before the first experiment.) Over a 40 minute period, sixteen lighted spots appear in irregular, randomly spaced positions. The subject signals the perception of the spots by pressing a key. The score consists of the number of spots correctly reported.

Flicker fusion. For this and the apparent motion tests, a stroboscope* was used.

The stroboscope consists of two glow modulator tubes (R 1130 B) acting as light sources and of five main parts—frequency generator, power amplifier, measuring unit, light box, and power supply equipment. The frequency generator is a conventionally coupled multivibrator that drives the power amplifier, a modified push-pull amplifier. The power amplifier feeds into a measuring unit with which, by throwing a switch, the current of both glow modulator tubes can be measured alternately. The measuring unit is connected to the light box which contains the two tubes, either of which can be disconnected for measuring the flicker fusion. The light from the lamps falls on a frosted glass plate. The time ratio of light to darkness (1:1) remains unchanged for the frequency range of 0.25 to 600 pulses per second. Scores represent the mean of five ascending and five descending trials, conducted in an "a-b-b-a" order.

Apparent motion. Two glow modular tubes with centers 64 mm. apart are arranged one above the other. When one of the lamps is illuminated, the other is ex-

*The apparatus employed in these tests and in the steadiness experiments was designed by Sture Borenius.

*Designed by Sven Lundquist and Chris Ottander.

Table I. *Results of performance tests with 8 subjects after the administration of single 1,200 mg. doses of emylcamate, meprobamate, and a placebo.*

Test	Mean scores			Units of scores	Direction of favorable effect: Higher score indicates	Probability of significant differences between paired compounds*		
	Placebo	Emylcamate	Meprobamate			Emylcamate-meprobamate	Emylcamate-placebo	Placebo-meprobamate
Simple reaction								
Lift time	226	215	243	Milliseconds	Decreased efficiency	95%		
Ballistic time	133	122	143	Milliseconds	Decreased efficiency	95%		
Total	364	343	390	Milliseconds	Decreased efficiency	99%		
Disjunctive reaction								
Lift time	381	367	388	Milliseconds	Decreased efficiency			
Ballistic time	221	202	262	Milliseconds	Decreased efficiency	99%		95%
Total	611	574	661	Milliseconds	Decreased efficiency	99%		
Tapping	25	24	24	Number of taps in five seconds	Increased efficiency			
Spoke's								
A (time)	125	123	140	Seconds for two runs	Decreased efficiency	95%		95%
B (time)	209	191	190	Seconds for two runs	Decreased efficiency			
B (errors)	2.3	1.8	1.7	Number of errors in two runs	Decreased efficiency			
Vigilance	8.8	8.7	6.7	Number of correct responses	Increased efficiency	95%		95%
Flicker fusion	42.3	41.1	41.9	Pulses per second	Increased efficiency			
Apparent motion	3.2	3.3	2.8	Pulses per second	Increased efficiency	95%		95%
Steadiness								
With support	8	5	12	Number of contacts	Decreased efficiency			
Without support	78	83	80	Number of contacts	Decreased efficiency			
Grip strength	32	34	33	Kilograms	Increased efficiency			

Emylcamate superior to meprobamate | No difference between emylcamate and placebo | Placebo superior to meprobamate

*95% = P < 0.05; 99% = P < 0.01.

tinguished. Five ascending and five descending trials were made. In the ascending series, the frequency is adjusted initially so that the test subject sees only two points not connected in space and then raised until the subject sees an oscillatory motion between the points as if the light were wavering between them. In the descending series, the initial frequency is so high that the subject sees two flickering points not connected in space. The frequency is then decreased until the oscillatory motion is again perceived.

Steadiness test. The test panel contains a series of holes, decreasing in size from 10.5 mm. to 2.5 mm. Five holes were used in this test.

The subject is asked to insert a round metal stylus 1 mm. in diameter into each of the holes and to hold it there for 5 seconds without letting it touch the sides of the hole. Scores represent the total number of contacts noted with the rim of the hole during four trials in each of the five holes.

This test was performed both *with support*, i.e., the wrist steadied by resting on

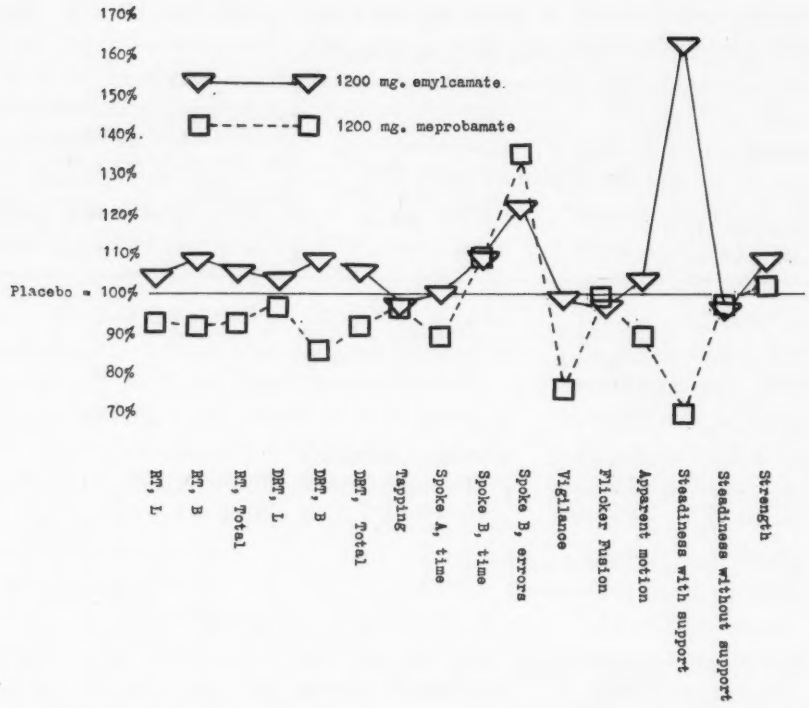

Fig. 1. The effects of single 1,200 mg. doses of emylcamate and meprobamate on performance of 8 human subjects. The results with emylcamate and meprobamate are expressed in terms of placebo performances. *(DRT)* Disjunctive reaction time; *(RT)* simple reaction time; *(B)* ballistic time; *(L)* lift time.

the table which holds the test panel, and *without support*, i.e., the arm free, not touching the table. As in other tests where motor response is measured, the hand favored by the subject is employed.

Grip strength. Grip strength was measured with an Aesculap dynamometer. The score represents the average of values recorded in five trials.

Results

The results obtained in the tests that employed 1,200 mg. doses of the drugs are given in Table I. The statistical test used was the analysis of variance.

Fig. 1 represents the data obtained with 1,200 mg. doses of emylcamate and meprobamate in terms of placebo performance.

A. Comments on the results of tests with 1,200 mg. doses. Appreciable differences were noted between the effects of emylcamate and meprobamate on performance. Significant differences were obtained in eight variables in which performance was more favorable with emylcamate than with meprobamate.

No significant difference in the effect in performance was noted between emylcamate and placebo.

Direct comparison of meprobamate with placebo revealed significant differences in three tests in which meprobamate had an unfavorable effect on performance.

In conclusion, *at the dose level of 1,200 mg.*, emylcamate and meprobamate have different effects on some functions measured, meprobamate having a depressant effect while no such decrease in efficiency of performance was found after administration of emylcamate.

It was thought desirable to increase the amount of emylcamate administered to tolerance in order to determine the maximum tolerated dose and also the most sensitive test variables. In the second part of the study, therefore, a single dose of 1,800 mg.

of emylcamate, meprobamate, or placebo, in coated tablets, was administered by the method described to 8 additional patients, 34 to 51 years old (average, 42).

The data are summarized in Table II. In Fig. 2, the results of the tests with emylcamate and meprobamate are expressed in terms of placebo performances.

B. Comments on the results of tests with 1,800 mg. doses. In seven of the sixteen variables, results obtained with 1,800 mg. doses show significant variations from those observed with 1,200 mg. doses. Data from this statistical analysis are not included here. In general, the larger doses tended to be more depressant.

With 1,800 mg. doses, both emylcamate and meprobamate impaired performance, although the two drugs showed considerable differences in effects from one test variable to another. With the increased dosage, significant differences between emylcamate and meprobamate were noted in only two of the sixteen tests, compared with in eight tests with the 1,200 mg. doses. In these two tests, emylcamate proved to be superior to meprobamate. Placebo produced significantly better performance than emylcamate or meprobamate in six and five variables, respectively.

Discussion

In Reitan's studies using Spoke's A and B tests, 1,200 mg. of meprobamate given 2 hours and an additional 400 mg. given 1 hour before the tests significantly impaired performance. However, lower doses, i.e., 400 mg. administered four times daily for 6 days and 2 hours before the tests, produced no significant changes in efficiency of performance. Kornetsky,[3] using a multiple stimulus-response apparatus, measured, in addition to other functions, simple motor responses that are somewhat analogous to reaction time. The administration of 800 mg. meprobamate 90 minutes before the test had no significant effect, while 1,600 mg. significantly lowered performance in these tests. Marquis and colleagues,[5] conducting similar experiments, found no significant alterations in the results of a steadiness test after the administration of 800 mg.

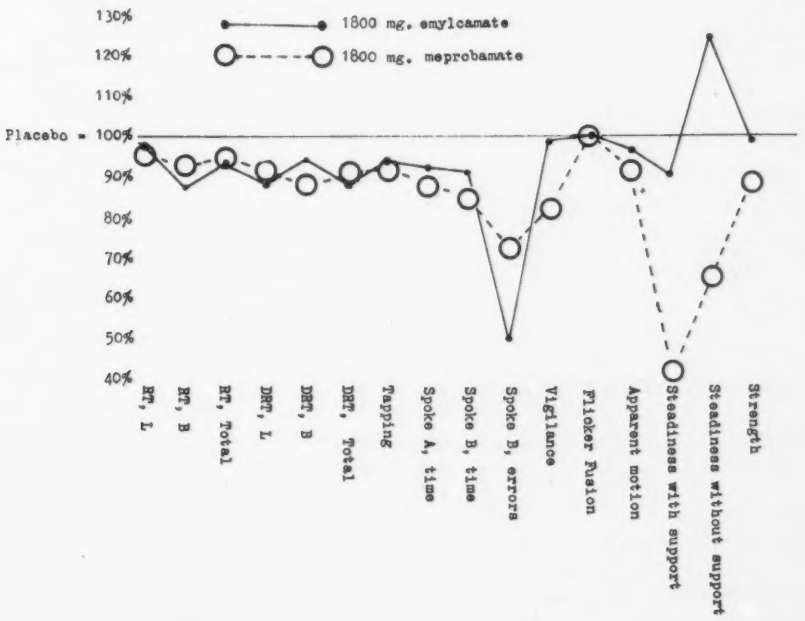

Fig. 2. The effects of single 1,800 mg. doses of emylcamate and meprobamate on performance of 8 human subjects. The results with emylcamate and meprobamate are expressed in terms of placebo performances.

meprobamate. Kelly and associates[1] found that administration of 800 mg. meprobamate twice daily had no definite effect on tapping rate, steadiness, flicker fusion, or apparent motion tests. According to the data in this summary of earlier experiments, impairment in some psychologic tests can be expected after 1,200 mg. of meprobamate, but a similar effect may not turn up at a lower dosage.

It is evident from Fig. 1 that 1,200 mg. meprobamate decreased efficiency of performance in our experiment. These findings are thus in accord with results reported in the literature. On the other hand, a single dose of 1,200 mg. emylcamate caused no impairment of performance in these tests. In doses of 1,800 mg. however, emylcamate also has a depressant effect.

The results obtained in these experiments allow us to draw some conclusions regarding the relative sensitivity of the different measurable variables to the effects of meprobamate and emylcamate and the suitability of such tests for evaluating these effects. Meprobamate, in both 1,200 mg.

Table II. *Results of performance tests with 8 subjects after the administration of single 1,800 mg. doses of emylcamate, meprobamate, and a placebo*

Test	*Mean scores*			*Units of scores*	*Direction of favorable effect: Higher score indicates*	*Probability of significant differences between paired compounds**		
	Placebo	*Emylcamate*	*Meprobamate*			*Emylcamate-meprobamate*	*Emylcamate-placebo*	*Placebo-meprobamate*
Simple reaction								
Lift time	217	224	227	Milliseconds	Decreased efficiency			
Ballistic time	159	184	172	Milliseconds	Decreased efficiency		99%	
Total	379	411	402	Milliseconds	Decreased efficiency		95%	
Disjunctive reaction								
Lift time	331	376	365	Milliseconds	Decreased efficiency		95%	
Ballistic time	224	237	255	Milliseconds	Decreased efficiency			
Total	562	640	627	Milliseconds	Decreased efficiency		99%	95%
Tapping	27	25	25	Number of taps in five seconds	Increased efficiency	Emylcamate superior to meprobamate	95%	99% Placebo superior to meprobamate
Spoke's								
A (time)	98	106	111	Seconds for two runs	Decreased efficiency			
B (time)	153	166	181	Seconds for two runs	Decreased efficiency	95%	Placebo superior to emylcamate	95%
B (errors)	1.0	2.0	1.3	Number of errors in two runs	Decreased efficiency		95%	
Vigilance	10.1	10.0	8.3	Number of correct responses	Increased efficiency	95%		95%
Flicker fusion	38.6	3.87	38.8	Pulses per second	Increased efficiency			
Apparent motion	2.3	2.2	2.1	Pulses per second	Increased efficiency			
Steadiness								
With support	6	7	15	Number of contacts	Decreased efficiency			
Without support	47	38	72	Number of contacts	Decreased efficiency			
Grip strength	36	36	32	Kilograms	Increased efficiency			99%

*95% = $P < 0.05$; 99% = $P < 0.01$.

and 1,800 mg. doses, is shown to significantly impair performance in the vigilance test even with the large variability of measurements. With 1,200 mg. meprobamate, performance deteriorates significantly in Spoke's A test and the apparent motion test; with a dose of 1,800 mg. the unfavorable effect of meprobamate is almost significant. Grip strength is affected by meprobamate at the 1,800 mg. level, which produces a significant decrease in skeletal muscle tone. The classic flicker fusion test was not affected by meprobamate or emylcamate even at the 1,800 mg. dose level.

In order to study the intercorrelations of the variables, the mean performance in each variable was calculated for each subject and rank correlations computed for all 16 subjects. The tests fell into four groups according to their intercorrelations, although border lines cannot be sharply delineated.

Group I: Lift and ballistic simple reaction times and lift disjunctive reaction time.

Group II: Ballistic disjunctive reaction time, tapping, Spoke's A and B tests, and vigilance.

Group III: Flicker fusion, apparent motion, and steadiness.

Group IV: Grip strength.

The orders of the tests could be systematically varied in the Latin square design. In the 1,200 mg. experiment, this opportunity to study the effects of different orders was used, the vigilance test being alternately placed in the first or the last part of the test battery, preceded or not preceded by the apparent motion and flicker fusion tests. The only significant effect was on steadiness with support, where the subjects obtained better results when they first had taken the vigilance test.

Summary

1. A battery of psychologic tests which includes sixteen measurable variables has been employed to determine the effects of large doses of meprobamate and of a new internuncial blocking agent, emylcamate, on the efficiency of performance in human subjects.

2. The 1,200 mg. and 1,800 mg. doses employed, given in a single administration, represent the usual therapeutic dosage of meprobamate for 24 hours and of emylcamate for 24 to 48 hours.

3. When compared with placebo, at 1,200 mg. dose levels, meprobamate impairs performance in several variables, while no such depressant effects were found for emylcamate. At the 1,800 mg. dose levels, both emylcamate and meprobamate impair performance.

Experimental design and statistical analysis by Stig Ek, Assistant Professor, Research Institute for National Defense, Sundbyberg 4. Mrs. May-Britt Lofvander performed the psychologic tests.

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The therapeutic value of sodium dextrothyroxine

Sodium dextrothyroxine has been shown to maintain athyreotic patients in normal metabolic status; dissociation of serum cholesterol reduction, basal metabolic rate, and heart rate from each other has been demonstrated. Reduction of serum cholesterol in all of 67 hypercholesterolemic patients was clinically significant and sustained. Cessation of angina pectoris in a myxedematous patient on substitution of dextrothyroxine for levothyroxine occurred. This was associated with correction of hypercholesterolemia and maintenance of a normal metabolic rate. High dosage of dextrothyroxine can produce hypermetabolism, but dosage sufficient to lower serum cholesterol in euthyroid patients did not result in increased basal metabolic rate. Sections of Lead II electrocardiogram records of 10 patients in whom significant serum cholesterol reduction was produced show clinically significant increase in heart rate in only 1 case. It is suggested that this lack of cardiac stimulation warrants a thorough clinical trial of sodium dextrothyroxine in cardiac patients.

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The postwar era of thyroxine chemistry begins with the discovery of triiodothyronine in 1951 by Gross and Leblond[1] and its subsequent synthesis by Pitt-Rivers in 1953.[2] A great many analogues of thyroxine have been found or produced since then and subjected to biologic study. The literature is extensive and growing rapidly. No attempt to review it will be made in this article.

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Modifications have been made in position and number of the iodine atoms in the molecule and changes in the composition of the side chain. The major alteration of the latter is deamination. Study of the former has revealed the fundamental importance of the position of the iodine atoms, illustrated by the completely different function of 3, 5, 3′-triiodothyronine, which is a superthyroxine, from that of 3′, 5′, 3-triiodothyronine, which is a thyroxine inhibitor. The biochemical law that biologic action is determined by molecular structure is manifest in the in vivo and in vitro trials of these substances. The concept presented to the American Therapeutic Society in 1958[5] that the analogues of thyroxine which have modified molecules must have various

functional effects has been advanced, if not established, by the comparison of the results of administration of some analogues with those of others and thyroxine itself. It was suggested that at least four or five biochemical systems of the body were affected in a spectrum of potencies by the different molecules derived from thyroxine. These biochemical systems control (1) heat production, (2) growth, (3) metamorphosis, (4) lipoid metabolism, and (5) nervous system activity. Doubtless other functions are similarly affected in different degrees. This is similar to the well-recognized variation in regard to gluconeogenesis, electrolyte metabolism, nerve physiology, hormone effects, and anti-inflammatory characteristics of the cortisone molecule and its analogues.

In the pursuit of these analogues, the simplest modification of all has been almost overlooked. This is spatial rearrangement to produce the dextroisomer. This form of thyroxine had long been considered to have little, if any, activity. Some careful animal studies showed no potency,[4] but clinical work indicated 10 per cent of the levothyroxine calorigenesis.[3] The production of abundant amounts of sodium dextrothyroxine, found free of the levoisomer,[6] permitted comparison[7] of the two in prolonged clinical trials of oral medication since, again contrary to earlier teaching, the sodium pentahydrate salt of thyroxine is well absorbed by mouth. These studies have shown the following facts about sodium dextrothyroxine:

1. It has about one-tenth of the calorigenic action of the levoisomer by weight.
2. It has the ability to lower serum cholesterol in the athyreotic patient without raising the basal metabolic rate.[8]
3. It is able to lower abnormal serum cholesterol in the euthyroid patient while maintaining the basal metabolic rate.[9]
4. It lowers serum cholesterol without stimulating the heart in either its athyreotic myxedematous or euthyroid state, whether the heart is normal or pathologic.
5. Preliminary evidence suggests that it can maintain a normal basal metabolic rate and serum cholesterol level without producing angina pectoris[10] in the patient with coronary arterial insufficiency.
6. Growth in athyreotic rats is maintained by tenfold dosage[11] as compared to levothyroxine.
7. Thyroid-stimulating hormone secretion of the pituitary is depressed to a considerable extent.[12]
8. Other endocrine effects, particularly in relation to androgen metabolism, are indicated.[11]

In short, rather than being an impotent shadow of levothyroxine, the dextroisomer is a thyroid hormone with useful therapeutic characteristics. The most significant of these are mild calorigenesis, strong cholesterol reduction, and weak catecholamine synergism. This suggests that total somatic substitution of dextrothyroxine in patients suffering from cardiovascular disease may be of value.

Clinical results

Calorigenesis in myxedema. This is illustrated in the case of R. V., a 40-year-old Mexican housewife, who developed spontaneous myxedema. The chronologic sequence is shown in Fig. 1. Before treatment, the serum PBI level was 0.8 μg per 100 ml., the serum cholesterol 495 mg. per 100 ml., and the BMR −36 per cent. Initial dosage of 1 mg. per day reduced the cholesterol to 310 mg. per 100 ml. without altering the BMR; gradual increase of dosage to a high level of 12 mg. per day produced a normal ECG, serum cholesterol of 210 mg. per 100 ml., and a BMR of −6 per cent. This dose later seemed to be slightly excessive and a maintenance dose of 8 mg. per day kept the cholesterol at 205 mg. per 100 ml. per day, ECG normal at 56 beats per minute, weight 156 pounds, and BMR −14 per cent.

Comment. This totally athyreotic myxedematous patient has been maintained in a euthyroid state on sodium dextrothyroxine pentahydrate alone for 11 months. Note the slow heart rate in the euthyroid state on 8 mg. of dextrothyroxine daily.

Low calorigenic characteristic. This trait may be compared in (1) a myxedematous patient and (2) a euthyroid patient.

1. M. B., who had untreated spontaneous myxedema, BMR −40 per cent, cholesterol 350 mg. per 100 ml., and a flat intercomplex ECG tracing, was treated for 33 days with a daily oral dose of 2 mg. of sodium dextrothyroxine. At the end of this time the BMR was still −40 per cent, the ECG interval base line still showed *no T waves,* and the serum cholesterol was reduced to 225 mg. per 100 ml.

2. B. N., having familial xanthomatosis, had PBI 5.2 μg per 100 ml., serum cholesterol 320 mg. per 100 ml., BMR −1 per cent, pulse 76, and weight 125 pounds. She was given progressively larger amounts of sodium dextrothyroxine daily by mouth. At the end of 9 months, during the last 3 months of which she was taking 16 mg. a day of this medication, the PBI was over 60 μg per 100 ml., the serum cholesterol was reduced to 220 mg. per 100 ml., the BMR was unchanged at −1 per cent, the pulse was 84, and weight was essentially the same, 124 pounds.

Comment. It must not be assumed from the identical basal metabolic rate values before and on 16 mg. of dextrothyroxine that the oxygen consumption of this euthyroid patient was not affected by the dextrothyroxine. It seems more probable that there was a *total somatic substitution* of the endogenous thyroid hormone secretions, levothyroxine and levotriiodothyronine, by the exogenous dextrothyroxine. The dose of 16 mg. a day, although large, is of the same order of magnitude as has been used

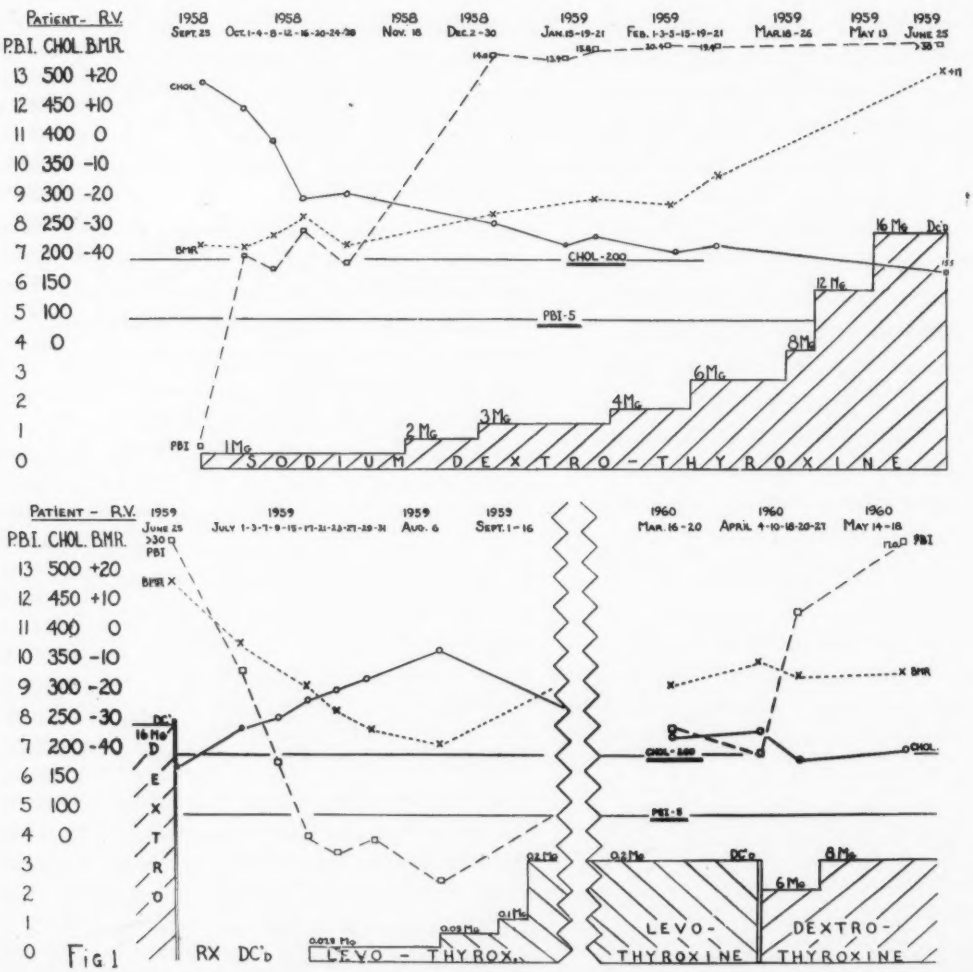

Fig. 1. Chronologic chart of metabolic and chemical tests during sodium dextrothyroxine and sodium levothyroxine medication in a case of spontaneous myxedema.

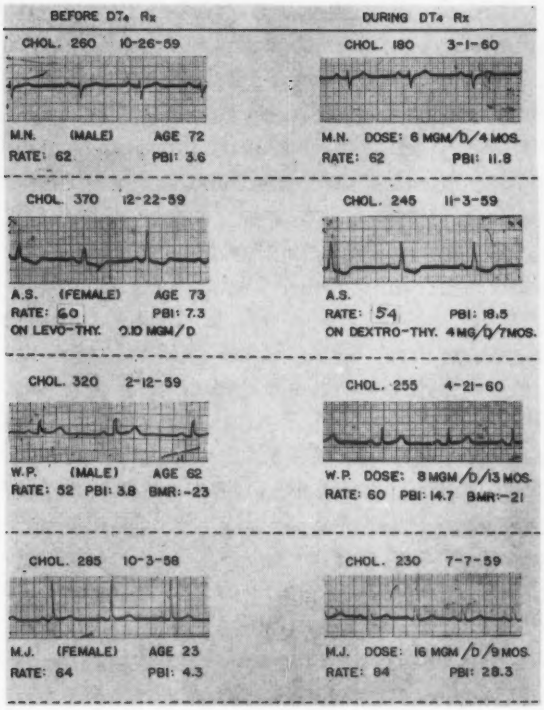

Fig. 2. ECG Lead II of patients before and during cholesterol reduction by sodium dextrothyroxine.

M. N., a senile man with benign prostatic hypertrophy, has hypothyroidism indicated by PBI level of 3.6 μg per 100 ml. Note slight changes on 6 mg. daily of sodium dextrothyroxine without change in heart rate. This suggests that hypothyroidism was corrected.

A. S., an athyreotic patient on 0.10 levothyroxine, has an ECG which also probably indicates hypothyroidism, since the cholesterol is 345 mg. per 100 ml. Angina, relieved by nitroglycerin, was occurring, to disappear on 4 mg. sodium dextrothyroxine daily.

W. P., a middle-aged minister, with no disease, evidently is slightly hypothyroid; the ECG shows some change.

M. J., a 23-year-old technician, shows increase in heart rate without change in voltage. No symptomatic or recognized physiologic change occurred.

in some patients with athyreotic myxedema to maintain normal metabolism.[6] One can expect then to find a dose in a euthyroid patient which will lower serum cholesterol without changing the final basal metabolic rate.

Lack of change in the electrocardiogram. This factor with euthyroid patients might be predicted from the lack of significant elevation or reduction of the basal metabolism in such cases. An analysis of the electrocardiograms of a series of patients in whom a significant reduction of serum cholesterol had been produced shows no significant alterations in rate, P-R interval, Q-T time or ratio, R or T wave potential, or S-T level, except those attributable to the correction of partial hypothyroidism (see Figs. 2 to 5).

Comment. It seems quite remarkable that there is so little significant change in the heart action under the influence of a thyroid hormone which is maintaining the basal metabolic rate and which has lowered the abnormally elevated serum cholesterol. This suggests the absence of effect on the sympathetic nervous system.

Hypothesis

In the 10 patients whose electrocardiograms were used for this study, reduction in serum cholesterol occurred; where hypothyroidism was present, results of increased voltage are indicated in the tracing during dextrothyroxine therapy, but increase in heart rate was only significant in 1 patient. Since pulse rate is based on the balance between vagotonic and sympathicotonic factors, it would appear that dextrothyroxine does not change this balance, i.e., does not potentiate the sympathicotonic forces. Further study of this aspect is certainly indicated.

Reduction of serum cholesterol

Reduction by sodium dextrothyroxine has previously been reported.[9] The present analysis extends that study to 67 patients and 22 normal subjects. Two-thirds of this series have been observed on sodium dextrothyroxine medication for 6 months or more and one-third for 10 months or more. Several patients have been treated for 2 years or more. The usual dosage ranges from 4 to 10 mg. a day. Continuing control of serum cholesterol without escape for long periods of time has been found. The effect on serum cholesterol in these two groups is summarized separately.

The patient group has the following characteristics:

1. Number: 67; 38 women and 29 men.

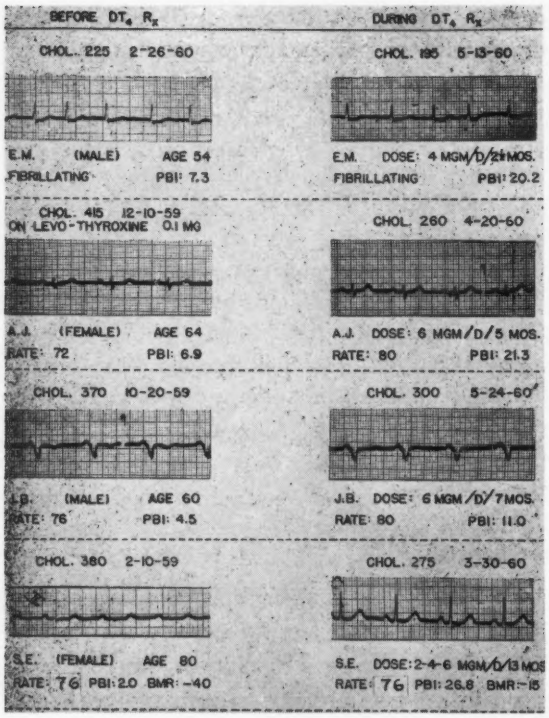

Fig. 3. ECG Lead II of patients before and during cholesterol reduction by sodium dextrothyroxine.

E. M. has severe rheumatic valvular heart disease, postcommissurotomy, and congestive failure. Some improvement in congestion during sodium dextrothyroxine medication occurred, raising the PBI to 20.2 μg per 100 ml. There was no change in ECG.

A. J., a patient with spontaneous myxedema, had improvement in cholesterol and ECG on dextrothyroxine.

J. B., a euthyroid patient with maximum myocardial infarction, ventricular aneurysm, and angina and hypercholesterolemia, showed gradual increase in sodium dextrothyroxine associated with moderate reduction of cholesterol, no significant ECG change, and improvement in exercise tolerance and angina, perhaps because of a better general regimen.

S. E., a senile myxedematous patient, was treated with sodium dextrothyroxine alone. ECG changes resulted from correction of myxedema.

2. Age: 12 to 83 years; 4 less than 30 years, 17 between 30 and 50 years, 31 between 50 and 69 years, 15 of 70 years or more.

3. Diagnoses: hypothyroidism 12, idiopathic hypercholesterolemia 25, diabetes mellitus 14, cardiac disease 6, xanthomatosis 5, hypopituitary 4, hypogonad 1.

4. Serum cholesterol level before treatment: range, 173 to 630 mg. per 100 ml.; 20 below 300, 34 from 300 to 400, inclusive, and 13 above 400 mg. per 100 ml.

The effect of sodium dextrothyroxine pentahydrate oral medication is given below.

The mean value of the serum cholesterol of the 67 patients before medication was 348 mg. per 100 ml. (standard deviation 89, standard error of the mean 10.9). The mean value during medication was 251 mg. per 100 ml. (standard deviation 64, standard error of the mean 7.8).

The p value of the difference between the means before and during medication was less than 0.001. The decrease from the premedication mean to the medication mean is 97 mg. per 100 ml., or 28 per cent. The level during therapy decreased from the original level to normal, i.e., 250 mg.

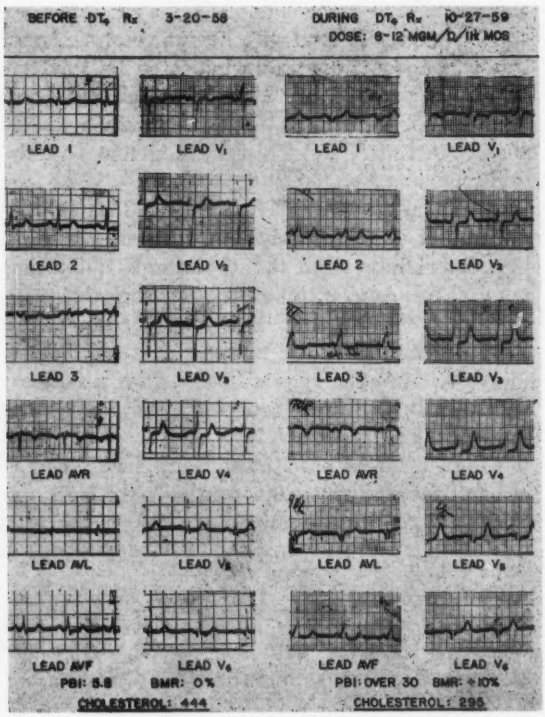

Fig. 4. In R. B., a 40-year-old female euthyroid patient with familial xanthomatosis and coronary arterial insufficiency, ECG changes were slight; increase in voltage, if significant, was due to overdosage with sodium dextrothyroxine; note PBI over 30 μg per 100 ml.

per 100 ml., or below in 42 cases, or about two-thirds of the series, *no matter how high the pretreatment level was.*

The mean decrease in the 20 patients with initial value below 300 mg. per 100 ml., the pretreatment mean being only 252.3 mg., was 52 mg., or 21 per cent; the mean cholesterol on medication was 200 mg. per 100 ml. The standard deviation and standard error of the mean of this group both before and during treatment are 34.5 and 7.8. The p value of the difference is less than 0.001.

The mean decrease in the 34 patients with initial values ranging from 300 to 400 mg. per 100 ml., inclusive, the pretreatment mean being 341 mg. and the mean on treatment being 259 mg., was 82 mg., or 24 per cent. The standard deviation and standard error of the mean of the two groups are, respectively, 24 and 4.1, compared to 46 and 7.9. The p value of the difference is less than 0.001.

The mean decrease in the 13 patients with high serum cholesterol values above 400 mg. per 100 ml., the pretreatment mean being 490 mg. and the mean on treatment 318 mg., was 172 mg., or 35 per cent. The standard deviation and standard error of the mean of the pretreatment and on treatment groups are, respectively, 61 and 17, compared to 75 and 21. The p value of the difference between the groups is less than 0.001.

It seemed that the effect of sodium dextrothyroxine in diabetics was greater than in the other patients no matter how high or low the initial serum cholesterol was. This is borne out by the statistical analysis of the values before and after treatment of the 14 diabetics. The mean value before dextrothyroxine medication was 347 mg. per 100 ml. and during this medication 229 mg. The difference is 118 mg. per 100 ml., or 29 per cent. The standard deviation and standard error of the mean of the two groups are, respectively, 102 and 27.3 and 61 and 16.4. The p value is less than 0.005.

It is most interesting that in a series of 22 normal young men and women with a mean pretreatment serum cholesterol value of 210 mg. per 100 ml., administration of an average dose of 6 mg. per day produced a reduction in only an occasional case. The on treatment mean cholesterol level was 190 mg. per 100 ml., a reduction of only 9 per cent, which has little statistical significance. This strongly suggests that the effect of the agent is only evident when an abnormal accumulation of serum cholesterol has occurred or when abnormality of synthesis is present, making it possible to inhibit the production to a certain degree. These relationships are not present in the normal person, with the result that no significant reduction occurs.

Absence of cardiotoxic effect

This trait of sodium dextrothyroxine, indicated above, makes it seem probable that the substitution of the dextroisomer in the tissues would ameliorate angina pectoris.

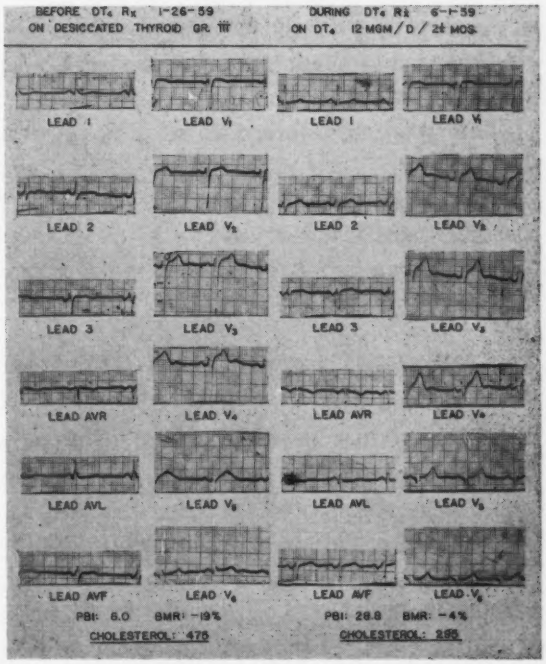

Fig. 5. W. C., an athyreotic man, 50 years old, with hypercholesterolemia of unknown etiology, exhibited changes which may be due to correction of occult hypothyroidism still present on desiccated thyroid 3 grains, with BMR −19 per cent and PBI 6 μg per 100 ml.

This is supported by the case of A. S.,[10] a 73-year-old myxedematous patient, who, as is not infrequent, could not take a large enough daily dose of either desiccated thyroid or sodium levothyroxine to become euthyroid because of the aggravation of angina pectoris. After gradual substitution of sodium dextrothyroxine, angina disappeared and the serum cholesterol became normal. When the dextrothyroxine medication was discontinued and the previous dose of levothyroxine reinstituted, angina occurred daily and continued until it was stopped and the sodium dextrothyroxine resumed. Angina occurred on a daily dose of 0.1 mg. of sodium levothyroxine with a serum cholesterol level of 345 mg. per 100 ml.; angina was absent on a daily dose of 4 mg. of sodium dextrothyroxine during a period of 7 months with a serum cholesterol concentration of 220 mg. per 100 ml. Lead II tracings on these medications are shown in Fig. 2 *(A.S.)*.

This may be interpreted as an indication that the dextroisomer of thyroxine does not synergize or potentiate the waves of endogenous catecholamines responsible for cardiac stimulation, as the levoisomer does.

The basal metabolic rate of A. S. on dextrothyroxine without angina pectoris was normal, —6 per cent; i.e., the absence of cardiac pain was not due to a lower level of oxygen metabolism than when she was on levothyroxine.

Conclusion

These studies indicate (1) that sodium dextrothyroxine has therapeutic usefulness in all forms of hypothyroidism, particularly the cases complicated by heart disease, (2) that it is an effective cholesterol-reducing agent in hypothyroid and idiopathic hypercholesterolemia and especially in diabetes mellitus, and (3) that an important field of clinical usefulness in heart disease is suggested by its ability to maintain normal oxygen consumption in a dose which reduces serum cholesterol without stimulating the heart through catecholamine synergism.

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The clinical pharmacology of the mercurial diuretic compounds

The organic mercurial compounds are the most potent diuretic agents in use. They act directly on the kidney to block the reabsorption of a portion of the filtered sodium chloride. The unreabsorbed salt then exerts an effect identical to osmotic diuretics; it limits water reabsorption augmenting urine flow. Within the kidney, the site of action is probably in the proximal segment, although direct evidence for this is lacking. Mercurial diuretics are administered as xanthine or monothiol complexes, in which form they are most rapidly absorbed and are least toxic. They are bound to renal cortical tissue and within minutes after injection are concentrated there to a greater degree than any other tissue of the body. They are secreted into the urine as cysteine complexes. Typically, they cause the loss of 2 to 5 L. of fluid that may contain more chloride than sodium ion. For this reason, repetitive use often produces a hypochloremic metabolic alkalosis. In this latter state, mercurials lose much of their diuretic potency. Treatment of the alkalosis with acidifying salts such as ammonium chloride restores their diuretic efficacy. This observation has been cited in support of the hypothesis that only in an acidic medium do organic mercurials release mercuric ion, their active principal. In refutation it has been observed that respiratory acidosis does not potentiate or restore the diuretic property of mercurials. An alternative hypothesis relates the mechanism of diuresis to the steric configuration of the compound.

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Edema is characterized by excesses of body water and sodium. The primary event is probably sodium retention followed by expansion of the volume of extracellular water.[13,44] Edema fluid collects in the extracellular space as an expression of the fact that the major portion of body sodium is restricted to this compartment. The quantity of sodium retained often exceeds that of water.[15,59] However, measurement of that readily sampled part of the extracellular fluid, the plasma, commonly reveals a low or normal sodium concentration. An explanation for this apparent paradox follows. A significant fraction of this abnormally retained sodium is sequestered in relatively avascular tissues such as cartilage and bone. In addition, some sodium leaves the extracellular fluid and enters cells in exchange for potassium. Thus the increase in total body sodium may exceed the increase in total body water and yet

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plasma sodium concentrations may be low.

No matter what the etiologic background of the process leading to edema, the retention of sodium is due to an alteration of renal transport mechanisms for sodium. In other words, enhanced reabsorption of filtered sodium is common to the edematous stage of cardiac, renal, or hepatic disease. Agents, such as the organic mercurials, that impair sodium reabsorption, thereby increasing sodium excretion, reverse the process. These agents are best called natriuretics. This name is literal; it describes their primary action. Of the diuretic agents in common use today, the organic mercurial compounds come closest to being true natriuretic agents. Their fundamental action is to inhibit the reabsorption of part of the sodium filtered at the glomeruli. The additional salt that remains within the tubule lumen then presents to more distal segments of the nephron more salt than it is capable of reabsorbing. This, in turn, restricts water reabsorption and results in an increase in urine volume. Thus, the organic mercurials are of interest to the physiologist studying ion transport processes, to the pharmacologist studying therapy, and to the clinician to whom they are the most potent diuretic agents available. Much has been learned about the organic mercurials since their accidental discovery 40 years ago. One would hope that this additional information has resulted in more effective therapy. The present article is not intended to be comprehensive but rather a review of recent work in this area, stressing those properties of the organic mercurial drugs that have been of interest to the author. For a more inclusive study of the subject, the reader is referred to recent review articles[18,38,43,46,50] or monographs.[44,56]

Localization of action

In 1920, a chance observation by an alert nurse led to the discovery of the diuretic effect of organic mercurial compounds.[49] Mercury in inorganic form had been used as a diuretic for centuries, but its toxic effects often produced a worse disease than the condition that prompted its use. It was soon appreciated that in an organic compound mercurials were effective and relatively safe. Within a brief period, several drugs were in common use, particularly for the treatment of congestive heart failure.

Mercurial diuretics were thought, at first, to act peripherally by mobilizing edema fluid. For this reason they were frequently administered by injection into the pleural space for the treatment of pleural effusion or into the peritoneal cavity for the treatment of ascites.[56] Govaerts[21] in 1928 and Bartram[3] in 1932 contributed the experiments that placed the site of action of mercury within the kidney. Govaerts removed a kidney from a dog at the peak of a diuresis produced by a mercurial and observed that this kidney continued to excrete large quantities of water when transplanted to another animal. In a more direct experiment, Bartram injected a mercurial diuretic into one renal artery of a dog and, collecting urine from the kidneys separately, observed a unilateral diuresis. Thus, the site of action of these diuretic compounds was localized to the kidney by direct proof.

Bartram's experiment, embellished with measurements of filtration rate and the excretion of sodium and mercury, is illustrated in Fig. 1.[43] Sodium excretion by the injected kidney was increased from 230 to 800 μEq per minute 30 minutes after the administration of Hg^{203}-labeled chlormerodrin* into the left renal artery. Simultaneously, the urine flow increased from a rate of 2.2 ml. per minute in the control periods to exceed 6 ml. per minute on the injected side. Although renal cortical tissue has a remarkable avidity for mercury,[22,60] some of the drug escaped from the injected kidney and was bound to and eventually produced a diuresis on the noninjected side. Additional features of this experiment will be discussed subsequently.

*Neohydrin.

Localization of action within kidney

The site within the kidney at which mercurial compounds exert their action has been the object of considerable study. Although direct proof is not yet at hand, the best evidence suggests that these drugs act within the proximal segment of the nephron. There have been two main routes of investigation in the solution of this problem. The first of these comprises observation of histologic or histochemical changes caused by mercury. The second has been to study changes in already localized renal functions that accompany mercurial diuresis.

Edwards[12] reported that toxic doses of inorganic mercury administered to rats produce microscopic lesions of proximal tubule cells. No changes were demonstrable with either inorganic or organic mercurials at dosages within the therapeutic range. Activity of the succinic dehydrogenase system of proximal cells is diminished by large doses of mercury, but, again, therapeutic doses fail to produce observable alterations.[47,57] A sensitive test of the number of protein-bound sulfhydryl groups within renal tissue was employed in a study by Cafruny, Farah, and DiStefano[9] before and after administration of mersalyl. These workers reported that mercury reduces the number of sulfhydryl groups not only in the proximal segment but in the loops of Henle and collecting ducts as well.

Of the functional studies performed in evaluation of the site of action of mercurials, the following are noteworthy. Giebisch[19] has proposed that the observed electronegativity of proximal tubular lumina is a consequence of active sodium reabsorption. The electrical potential of proximal lumina of rats is diminished by pretreatment with chlormerodrin. That the potential is only diminished and not abolished is consistent with the observations that the mercurials block only a fraction of the sodium reabsorptive process.

Certain functions, known by the precise technique of micropuncture to reside within the proximal segment, are depressed by mercurials. For example, the maximum tubular capacity to reabsorb glucose[23]

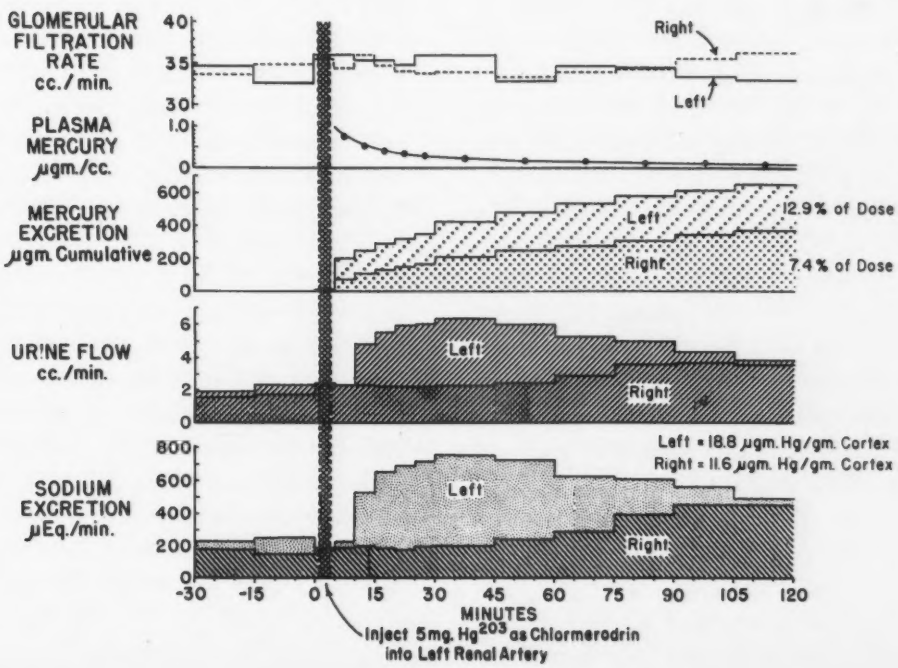

Fig. 1. Some effects of intrarenal arterial injection of chlormerodrin.[43]

and to secrete *p*-aminohippuric acid[5,8] is transiently lessened in man during a mercurial diuresis. The validity of this argument as a localization technique is considerably lessened by the following observations. Both man and dog respond to a mercurial diuretic with essentially identical patterns of water and electrolyte excretion. But, unlike man, neither glucose reabsorbtive[28] nor *p*-aminohippuric acid secretory ability[5] is affected by 2 mg. per kilogram of mercury in the dog. However, toxic doses of mercury will cause glycosuria in the dog.[24]

The stop-flow technique[37] provided a new approach to the problem of localizing the site of action of mercurials. These experiments are performed during an osmotic diuresis produced by infusion of 20 per cent mannitol in saline. One ureter is clamped and the kidney becomes progressively distended with glomerular filtrate until such time as intrarenal pressure equals the filtration pressure. This occurs within 1 or 2 minutes at the high rates of urine flow obtained with a 20 per cent mannitol infusion. Filtrate trapped within the lumina is subjected to the modifying action of the epithelial cells for as long as the ureter remains clamped. This accentuates renal reabsorptive and secretory effects as the duration of contact of fluid with tubule cells is prolonged. After 6 to 8 minutes of urinary stasis, the ureteral clamp is released and fluid flowing from the kidney in the ensuing 2 minutes is collected serially in about thirty to forty 1 ml. samples. The first five to ten of the samples derive largely from the distal segment, whereas later samples derive from the proximal segment. These serial samples are then analyzed for creatinine, which serves as an index of water reabsorption, and for *p*-aminohippuric acid, which marks proximal segment activity in those samples in which its concentration is highest. Kessler and colleagues[28] observed a doubling of the sodium/creatinine clearance ratio in fluid from the proximal segment by the mercurial diuretic chlormerodrin. Sodium clearance ratios of samples derived from the distal segment were unaltered by mercury. The above data were confirmed by Vander and associates[55] and are interpreted as indicating a proximal site of action of mercury.

However, suggestive stop-flow and other experimental results are in localizing the site of action of mercury; the data provide only indirect evidence. The definitive experiment will identify the ion transport mechanism or mechanisms sensitive to the organic mercurial diuretic compounds.

Route of administration

The mercurial diuretics must, in many instances, be administered parenterally to be effective.[16] Herein is their major disadvantage. Although some patients tolerate and respond to oral preparations, the effective dose is high. Moyer, Handley and Wilford,[41] in a 2 year study of patients with congestive heart failure, observed that the therapeutic dose ranged from 420 to 560 mg. Hg per week. These authors estimate that the oral dose of the most effective drug, chlormerodrin, is the equivalent of 80 to 160 mg. Hg per week of meralluride* injected intramuscularly. Diarrhea frequently accompanies the use of mercurials by the oral route. Perhaps this is an expression of the fact that more than 90 per cent of mercury so administered is excreted in the feces.[25] Any advantage that existed to the use of orally effective mercurials has been lost to chlorothiazide.[32]

Most commonly, mercurials are administered by intramuscular injection. By this route, many of these drugs cause local pain and varying degrees of tissue reaction. The addition of xanthines such as theophylline to certain of these mercurials decreases this local reaction and speeds absorption from the side of injection.[11] In addition, and possibly associated with these effects, theophylline also decreases the cardiac toxicity of organic mercurials. Modell and Krop[40] observed that the lethal dose of mersalyl†

*Mercuhydrin.
†Salyrgan.

in cats is approximately 16 mg. per kilogram of body weight. When theophylline is added to mersalyl in equimolar quantities, the lethal dose is increased by a factor of two. Curiously, the toxicity of inorganic mercurial diuretics ($HgCl_2$) is unaffected by theophylline.

Monothiol compounds also reduce toxic actions of mercurials[14] and enhance their absorption.[34] These sulfur-containing compounds complex with both inorganic and organic mercurials according to the following reaction[33]:

$$\text{R-Hg-X} + \text{R}^1\text{-SH} \longrightarrow \text{R-Hg-SR}^1 + \text{HX}$$

Use has been made of this in the production of mercurial drugs that possess new and beneficial properties. The addition of a mercaptide to the organic mercurial component of mercurophylline results in the sulfur-containing diuretic, mercaptomerin.* This drug is sufficiently nontoxic to permit subcutaneous administration. Evidence that monothiols such as the above increase the speed of absorption may be inferred from the observation that diuretic response to mercaptomerin is equally rapid when administered by subcutaneous and intravenous routes.[23] Cysteine complexed with $HgCl_2$ transforms this inorganic salt from a highly toxic and poorly diuretic agent to one of lesser toxicity that exceeds many of the organic compounds in diuretic potency.[35]

The intravenous route of administration is most certain to deliver mercurials to their site of action. All problems of absorption, particularly from edematous tissue, are avoided. However, one of the most serious toxic reactions to mercury, ventricular fibrillation, has been reported only after intravenous administration.[26] Although this often fatal reaction is rare, there seems to be little justification for intravenous use. It has been suggested that patients unresponsive to mercurials administered by intramuscular injection will diurese with the same dose given intravenously.[20] This has been questioned by other workers who report that intramuscular and intravenous therapy produce identical diuretic response.[39] As mentioned previously, the speed of onset of diuresis with mercaptomerin is the same whether administered by the intravenous or subcutaneous route.[23] There exist many alternative means of inducing diuresis in the patient refractory to intramuscularly injected mercurials. Kwit and co-workers[31] observed that frequency of administration was often related to success of therapy. In this study, 90 per cent of patients with congestive heart failure were maintained at dry weight by the use of mercurials daily. Only 50 per cent of the control group of patients were maintained at dry weight when treated with periodic injections. In addition, the refractory patient may be treated with other agents that potentiate the action of mercurials (vide infra).

Distribution and excretion

Mercurial compounds disappear rapidly from the plasma after intravenous administration. Although bound to plasma proteins,[33] they are concentrated to a remarkable degree by renal cortical tissue.[6,22,30] Following the formation of complexes with naturally occurring monothiols,[58] they are secreted into the urine.[6]

Threefoot and associates[54] studied the plasma concentration time course of radiomercury-labeled meralluride in humans after intravenous administration. These workers observed that the plasma radioactivity falls off rapidly in the first 5 to 10 minutes. Thereafter, the rate of fall-off is considerably reduced and, by 30 minutes after injection, the rate of disappearance of radiomercury is quite slow and constant. The regression of plasma radiomercury may be resolved graphically into three dominant time components. The above authors suggest that the most rapid component might be mixing of radiomercury with plasma. The slowest component corresponds in time to urinary excretion of mercury, and the intermediate time com-

*Thiomerin.

ponent probably reflects distribution and binding of radiomercury to tissues. Weston and co-workers[60] demonstrated the binding of mercury to human renal tissue by the following technique. Several minutes after the intravenous administration of a mercurial diuretic, renal venous and arterial bloods were sampled simultaneously and analyzed for mercury. With this information plus the rate of urinary mercury excretion, they calculated that the rate of extraction of mercury from the plasma by the kidney exceeded the rate at which mercury was lost in the urine. They concluded that mercury is bound to renal tissue.

The above findings in humans have been confirmed by more direct experimental techniques in the dog (see Fig. 1). Borghgraef, Kessler, and Pitts[6] confirmed the work of Threefoot and colleagues[54] by demonstrating that (1) the rapid components of simultaneously administered Evans blue and mercury are almost identical, (2) pretreatment with dimercaprol, an agent known to increase mercury permeability of tissues,[14,33] markedly shortens the intermediate or tissue-binding component, and (3) nephrectomy slows the disappearance of mercury from the plasma only after the phases of mixing and tissue binding. Table I summarizes their data on the fate of intravenously administered chlormerodrin.

The excretion of mercury must involve several steps, since almost none is filtered by the glomeruli.[30] The mercurials, complexed with the sulfhydryl-containing constituents of the plasma, are delivered to renal cells via the peritubular blood supply. The remarkable avidity of renal cortical tissues for mercury in many chemical forms suggests that this tissue possesses a specialized system for concentrating the metal. That excretion occurs without filtration suggests tubular secretion. Further evidence for secretion derives from the fact that both mersalyl and meralluride are excreted as cysteine or acetylcysteine complexes.[58] Binding and possibly excretion of mercury involve active transport in the sense that they utilize metabolic energy to move mercury against concentration gradients. Fig. 1 illustrates some of the above-mentioned features. Note that some of the mercury injected into the left renal artery was bound by cortical tissue. At a time when the plasma mercury concentration measured 0.1 μg per milliliter, the left cortex contained 18.8 μg per gram. Not all of the injected mercury was bound to renal tissue, however. That which escaped binding by the left kidney entered the general circulation, resulting in a plasma concentration of about 1 μg per milliliter. The mercury left the plasma rather rapidly during the next 15 to 20 minutes as both kidneys extracted the drug from the blood perfusing them. At the termination of the experiment, saline diuresis was evident on both sides, and the noninjected kidney had concentrated mercury from the plasma by a factor of more than 100. The cumulative excretion of mercury was 20.3 per cent of the total dose in 2 hours. Excretion of mercury by the two kidneys was roughly in proportion to their respective cortical concentrations of it.

Table I. *Tissue distribution and renal excretion of mercury after the intravenous administration of chlormerodrin*[6]

Minutes after injection	*Percentage of total dose*			
	Kidney	*Urine*	*Other tissues*	*Plasma*
10	10	4	65	21
40	25	15	50	10
120	30	30	37	3

Diuretic actions

The response to therapeutic doses of mercurial diuretics depends upon the patient's extracellular fluid reserve and renal function as well as frequency and route of administration. The former variables make quantitation of response most difficult. Bioassay of any diuretic is adequate insofar as one can choose comparable patient populations and select the most appropriate parameters to gauge response.[17,29] Typical response in man to 40 to 80 mg. of mer-

cury (1 to 2 ml. of the more common drugs) administered parenterally usually follows this sequence.[44,56] Diuresis begins within 2 hours, is maximum in about 5 hours, and lasts with diminishing effect for about 24 hours. Optimal fluid loss may be achieved by bed rest during the diuretic period. The urine volume is typically increased by 2 to 4 L. during the day after mercurial injection. Reports of the loss of as much as 15 L. from a single injection probably reflect effects other than those ascribable to direct mercurial action. Such a response might result from reduction in venous pressure or plasma volume secondary to the more modest diuresis caused by mercury. Too vigorous diuresis may result in dehydration, particularly in the elderly patient. Repetitive use of mercurials may lead to a metabolic alkalosis (vide post). By the nature of the processes leading to diuresis, adequacy of glomerular filtration rate is a determinant of response. Equally important are the volume and character of the extracellular fluid.

It is accepted that mercurial diuretics block reabsorption of a portion of the filtered sodium chloride.[13,44,59] Reduction of filtration rate, then, reduces the load of salt delivered to proximal tubule cells. This, in turn, diminishes the ability of the kidney to excrete salt and water. Selkurt, Hall, and Spencer[52] effected variable reduction in the filtration rate of dogs by compressing the aorta above the renal arteries. Sodium excretion was almost abolished when filtration rate was lessened by 50 per cent. In comparable experiments performed in the course of a mercurial diuresis, Pitts and Duggin[45] observed a 90 per cent reduction in sodium excretion accompanying a 25 per cent reduction in glomerular filtration rate. Davidson, Levinski, and Berliner[10] succeeded in placing an inflatable cuff about one renal artery and, thus, were able to cause small but graded reductions in renal blood flow and filtration rate. Their studies revealed that decrements in filtration rate resulted in equal decrements of sodium excretion.

The quantity and composition of the extracellular fluid also affect the response to mercurial diuretics. Delivery of sodium chloride to the kidney depends upon both the extracellular fluid (plasma) concentration of these ions and their filtration. It has been observed many times that edematous patients treated repeatedly with mercurials develop a metabolic alkalosis.[18,38,43,50] This extracellular fluid derangement is characterized by elevated plasma bicarbonate and reduced plasma chloride concentrations. Coupled with this state is a diminished response by the patient to mercurial diuretics. Hypochloremic alkalosis reflects the fact that mercurials increase the excretion of more chloride than sodium ion.[46] Ammonium chloride[48] and more recently lycine hydrochloride[36] correct the alkalosis and restore the extracellular fluid chloride concentration and the patient's response to mercurials.

The effect of mercurials to produce urine containing more chloride than sodium ion has been used to advance two hypotheses pertinent to this article. The first of these states that mercurials block not sodium but chloride reabsorption and that this explains the predominance of urinary chloride.[51] The second hypothesis, to be discussed in a subsequent section, states that mercurial diuretics are acid labile. In an acid medium, organic mercurials release minute amounts of their active principle, mercuric ion, at their site of action.[35,42]

There is no proof that sodium ion is actively transported in the proximal tubule. Presumptive evidence favors sodium over chloride ion, however. Electronegativity of the proximal lumen is best explained by active cation reabsorption and passive diffusion of anion along an electrical gradient.[19] There is additional evidence for the primacy of sodium ion transport to be found in other tissues.[44] The excretion of more chloride than sodium in mercurial diuresis does not necessarily indicate that active chloride reabsorption has been inhibited. Berliner[4] states that if sodium reabsorption is blocked in the proximal seg-

ment, greater quantities of this ion will be presented to more distal segments of the nephron. Distal sodium reabsorption occurs by exchange of this ion with cellular stores of hydrogen or potassium ion. This latter process is very slightly affected by mercurials. Augmented delivery of sodium to this segment enhances cation exchange, and tubular fluid sodium is replaced by potassium or hydrogen ion. Chloride ion, unaffected by the exchange process, appears in the urine in greater concentration than sodium ion.

The excretion of water during a mercurial diuresis is secondary to the excretion of ions. This was demonstrated by Brodsky and Graubarth[7] in a comparison of mannitol with mercurial diuresis in dogs. They observed that the rate of urine flow depended upon the urinary osmotic load regardless of whether the load was increased by mercurials or mannitol. Spritz and colleagues[53] came to the same conclusion in a study of the response of patients in congestive heart failure treated with mercurial diuretics. Under these conditions of diuresis, urine flow was directly proportional to osmolal clearance.

The data and interpretations referred to above concern the transient effects of mercurials. As the circulatory status of the patient improves with diuretic therapy, salt and water excretion may proceed independent of the immediate actions of mercurial diuretics.

Mechanism of action

The potentiating action of ammonium chloride on a mercurial diuresis was discovered shortly after the introduction of merbaphen.*[27] The effect of this salt is so marked that, quite logically, it has been assumed by many to be a key to the mechanism of the diuretic action of mercurials. The explanation offered is that the pH of body fluids is altered or that redistribution of ions results from ammonium chloride administration. Commonly, both of these events result from the use of this salt in clinical practice. Occasionally, the plasma pH or chloride concentration changes are so small that they are difficult to measure. The better criterion, at least in the circumstances of hypochloremic alkalosis, appears to be the urinary chloride concentration.[48] The patient resistant to mercurials may be successfully treated after a course of ammonium chloride has increased the urinary chloride concentration to 40 mEq. per liter. One may summarize these observations as follows: ammonium chloride potentiates and sodium bicarbonate weakens a mercurial diuresis.

Table II. *A comparison of the chloruretic effect of an inorganic and organic diuretic mercurial under varying conditions of acid-base balance.*[42] *$\Delta U_{Cl}V$ mEq. per kilogram every 3 hours*

Diuretic	*Acidosis*	*Normal*	*Alkalosis*
Mercuric cysteine	8.5	6.5	3.6
Meralluride	3.4	0.9	0.2

How do altered body pH and ion distribution affect organic mercurials? Mudge and Weiner[42] offer this explanation: Organic mercurial compounds are acid labile. Although not ionizable in the usual sense, mercury separates from the parent compound in a strongly acid medium. The monothiol cysteine facilitates rupture of a mercury-carbon bond, a feature common to all organic diuretic mercurials. This group of investigators compared the effects of meralluride with mercuric chloride complexed with cysteine under varying conditions of acid-base balance. Table II summarizes the results after administration of 1 mg. Hg per kilogram in these two forms. They conclude that the potency of organic mercurials is considerably more pH dependent than that of inorganic mercury.

In refutation it may be cited that meralluride is not potentiated by acidosis that

*Novasurol.

Table III. *Some effects of HCO_3^- infusion and CO_2 inhalation on mercurial diuresis. See text for mode of presentation of data*

Date	V (ml. per min.)	pH		pCO_2		HCO_3^-		Na^+		Cl^-	
		Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine
NaCl											
Control	3.6	7.41	7.06	28	32	16	97	147	98.2	121	97.8
Experimental	6.1	7.40	6.17	27	21	15	99	148	92.5	124	90.2
								Δ − 5.7		Δ − 7.6	
HCO_3^-											
Control	3.8	7.51	7.81	46	55	31	81	150	95.4	105	98.8
Experimental	4.1	7.52	7.78	41	54	30	83	151	94.8	107	98.2
								Δ −0.6		Δ −0.6	
12 per cent CO_2											
Control	2.0	7.14	7.28	106	128	35	95	147	97.2	106	98.9
Experimental	4.2	7.20	7.06	107	118	36	93	149	93.6	106	95.2
								Δ − 3.3		Δ − 2.6	

results from inhalation of 6 to 8 per cent carbon dioxide.[1] Additional data are presented in Table III.* In these experiments, dogs were infused with 1 L. of isotonic saline or $NaHCO_3$ while breathing either room air or 12 per cent carbon dioxide. On reaching a stable urine flow rate, control periods were obtained and 1 mg. Hg chlormerodrin per kilogram was given intravenously. The table includes the results obtained in the control periods and in periods of maximum diuresis. A minimum of three studies was performed under the four experimental conditions. Plasma ion concentrations are expressed in milliequivalents per liter, whereas the urinary data are expressed as percentage of the filtered ion that was reabsorbed. This latter calculation has the virtue of correcting urinary ion excretion for variations in filtration rate.† Normally, over 99 per cent of the filtered sodium and chloride is reabsorbed. Reabsorption of these ions decreases slightly in saline-loaded animals. Mercury depressed the reabsorption of 7.6 per cent of the filtered chloride and 5.7 per cent of the filtered sodium. This reflects the fact that plasma contains three-fourths as much chloride as sodium.

Bicarbonate infusion resulted in a metabolic alkalosis accompanied by an evident decrease in plasma chloride concentration. The urinary response to the same dose of mercury was almost abolished. This is comparable to the state observed in patients treated repetitively with mercurials. Acidosis produced by the inhalation of 12 per cent carbon dioxide did not potentiate the diuresis. Indeed, the response is diminished. Since carbon dioxide is freely diffusible in all body fluids, it is reasonable that carbon dioxide acidosis is intracellular as well as extracellular. Inhalation of carbon dioxide in this concentration increased the plasma bicarbonate and reduced the plasma chloride concentrations to the same extent as did the infusion of bicarbonate. Although the diuresis during breathing of carbon dioxide was not as voluminous as with a saline load, neither was it as meager as with a bicarbonate load. It seems fair to conclude that acidosis

*R. H. Kessler, O. P. A. Heidenreich, F. Krück, and R. F. Pitts: Unpublished observations.

†Filtration rate × Donnan factor × plasma ion concentration = $\text{Load}_{\text{ion}} \cdot \frac{\text{Load}_{\text{ion}} - (U_{\text{ion}}V)}{\text{Load}_{\text{ion}}} \times 100 =$ Percentage of ion filtered that is reabsorbed.

per se is not the determinant to the success of mercurial diuresis.

Heavy metals such as mercury have an affinity for sulfhydryl groups of proteins and protein constituents. Organic mercurial compounds such as *p*-chloro-mercuri-benzoate and methyl mercury are used as inhibitors of sulfhydryl-containing enzyme systems. A logical extension of this reasoning is that mercurial diuretics act by inhibiting sulfhydryl enzymes concerned with the energy supply or carrier system of ion transport. Neither of the in vitro sulfhydryl inhibitors mentioned above act as diuretics.[30] *p*-Chloro-mercuri-benzoate, like the diuretic mercurial compounds, is concentrated in renal cortical tissue and is secreted into the urine. Renal binding and secretion of mercury are not criteria of diuresis. In what ways does this aromatic mercurial differ from the diuretic compounds?

All of the diuretic drugs in use today have the following basic structure:

$$R\text{-}CH_2\text{-}CHOCH_3\text{-}CH_2\text{-}Hg\text{-}$$

The R substituent is variable in size and constituency but in each instance is hydrophyllic. In a study of three clinically useful diuretics and six structural variants of these, Kessler, Lozano, and Pitts[30] concluded that the steric relationship of mercury to the remainder of the molecule might be a determinant of diuretic action. For example, $HO\text{-}CH_2\text{-}CH = CH\text{-}Hg\text{-}$ had diuretic properties but not $HO\text{-}CH_2\text{-}CH_2\text{-}Hg\text{-}$, the two carbon analogue of this alcohol. Possibly, the reaction of mercury with a cellular component at the site of its action depends on a two point association. Mercury might be one point and the hydrophyllic group, three carbons distant, the other point of reaction. There are exceptions to this intact molecule hypothesis within the aromatic group of mercurial compounds. There are also exceptions to the hypothesis that diuresis is associated with acid lability and release of mercuric ion at the site of action. The final answer will add to the rational use of these diuretic agents.

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Clinical pharmacology of antihypertensive agents

A review is presented of the pharmacologic action of the drugs used in the management of hypertensive patients. The actions of these drugs as established in laboratory studies are correlated with experiences based on their use in the clinic. The role of drug therapy in the treatment of hypertension is evaluated in terms of the underlying functional pathologic background of the disease. As a basis for rational therapeutics, the effectiveness of the available drugs and their practical value are examined in the light of their pharmacologic action in man.

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The treatment of hypertension has undergone radical change during the last two decades. From the attitude that the disease constituted an inevitable consequence of the aging process and hence was not amenable to therapy, knowledge has grown until this common disorder is now treated by surgical and dietary measures as well as by a variety of drugs which are capable of reducing the blood pressure. The latter may be classified into two groups according to their mode of action:

1. Drugs which modify the neural mechanisms that maintain the blood pressure at its elevated level. These act either to moderate stimuli from the higher centers which exert potent effects in raising the blood pressure or to inhibit the normal action of the sympathetic in maintaining the tone of the cardiovascular system. The latter group of drugs either acts within the central nervous system, blocks the transmission of nerve impulses at autonomic ganglia, or inhibits the action of postganglionic sympathetic nerves.

2. The natriuretic agents which reduce the extracellular fluid volume and alter the water and electrolyte pattern of the tissues.

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General considerations

A consideration of the nature and the pathogenesis of hypertension and the beneficial effects to be derived by lowering the blood pressure is essential for any intelligent approach to the therapeutic use of antihypertensive drugs. There is good evidence to indicate that hypertensive cardiovascular disease is of renal origin, although the nature of the renal disturbance responsible for the disease is still a matter to be established with certainty. The term "hypertension" or, more correctly, "hypertensive cardiovascular disease" may be defined to designate a disease entity rather than simply an abnormal increase in arterial blood pressure, as is frequently done.[22,23,25]

All of the drugs presently used in the

treatment of hypertensive cardiovascular disease act simply to alter homeostatic mechanisms which maintain the blood pressure at its abnormally high level. These drugs accordingly cannot be considered as rational in the sense in which this term is used in therapeutics. A rational treatment of hypertension would involve the use of an agent which actually countered or corrected the basic defect and thereby relieved the increased peripheral resistance characteristic of the disease. Such a drug would lower the pressure without the deleterious effects induced by most of the presently available agents which lower the blood pressure.

Effects of lowering the blood pressure. The arterial blood pressure is of necessity a labile function since it must vary in response to the constantly occurring alterations in the demands of the organism for an adequate blood supply to the tissues. The elevated blood pressure of the hypertensive patient is even more labile, responding to a variety of influences, and is lowered by many drugs which affect the blood pressure of the normal individual to only a slight degree. When such lowering results in a deficient blood supply to the heart, kidney, brain, and other vital organs, it results in the same adverse effects as occur when the blood pressure is lowered in the normal person. It is not surprising, therefore, that many drugs effective in lowering the blood pressure in experimental preparations should prove unsuited for clinical use. Even in the case of the drugs which have come into clinical use for the treatment of hypertension, the lowering of the blood pressure often induces these same evidences of a reduced circulation through the brain, kidney, heart, and other tissues and may induce fatal effects as well as such less serious symptoms as postural hypotension, dizziness, etc., which limit their use to doses inadequate for reduction of the blood pressure to the desired degree. The drugs most commonly used, therefore, are those which are limited in hypotensive action, such as reserpine and the saluretic drugs. They are less likely to induce the alarming symptoms caused by the more effective agents, which are added to the therapeutic regimen only in the more seriously affected patients.

In addition to the drugs in use at present, which are to be discussed later, others which in the laboratory exert a hypotensive effect have received preliminary clinical trial. These include the amine oxidase inhibitors, serotonin and aldosterone antagonists, inhibitors of the metabolic decarboxylation of dihydroxyphenylalanine and 5-hydroxytryptophan, etc. That such diverse compounds as well as others that have a variety of effects on the basic enzyme systems of the body should lower the blood pressure of the hypertensive patient is not surprising. Their other effects as well as their unwanted side effects should temper any undue optimism as to their ultimate clinical usefulness. Only prolonged careful and critical study on large groups of patients can determine their ultimate value, if any, in the management of hypertension.

Relation of animal to human studies in hypertension. Certain unique features differentiate the pharmacologic effects of antihypertensive drugs in the laboratory from their action in the clinic and must be considered in order to appreciate some of the inconsistencies prevalent in this field of investigation. Although it has been assumed generally that hypertension as produced experimentally in the laboratory and as it occurs spontaneously in the human are different entities, there is no basis in fact for this view. If definite criteria are adopted for defining "hypertension" rather than using the term simply as synonymous for an elevation in blood pressure, one finds that hypertension induced experimentally in the animal manifests the same clinical, hemodynamic, and pathologic features as it does in the human and that there is every reason to accept the two as counterparts of the same fundamental disease.[22,23,25]

Were a drug available which acted to overcome the basic disturbance responsi-

ble for the elevation in blood pressure, one would anticipate that such a drug would exert comparable effects in the experimentally hypertensive animal as in the human. On the other hand, it is not surprising that drugs that act in a nonspecific manner should manifest diverse effects in man and animals and that pharmacologic studies on animals are not reliable in predicting the blood pressure–lowering effect of certain drugs in man. This is true, for example, in the case of inhibitors of monoamine oxidase, aromatic amino acid decarboxylase, and α-methyl dopa.

Certain major discrepancies between the effects of hypotensive drugs as used in the clinic and their action in the animal rendered hypertensive are readily explicable. For example, drugs which act by blocking the sympathetic nerves (ganglion-blocking agents, guanethidine, bretylium tosylate, etc.) act primarily to induce postural hypotension, and their chief blood pressure–lowering action is evident in man mainly only in the upright position. One would not expect these to exert an effect in the rat or dog, as indeed is the case. On the other hand, drugs, such as the natriuretic diuretics, which act by decreasing the blood volume should be as effective in the rat as in the human, and this likewise is the case.

One can utilize the study of antihypertensive drugs in hypertensive animals with great profit in evaluation of their potential effectiveness in man. It is unfortunate that such studies have been meager because of the difficulty of maintaining a colony of hypertensive animals as well as the assumption that the two disorders are unrelated. Where they have been carried out, there is the anticipated correlation between the two types of study, and conclusions drawn from simple and easily performed experiments in the laboratory have proved much more accurate in evaluating the potential value of a given drug than the pharmacologic data derived by other techniques or obtained by inadequately controlled clinical data. The overly optimistic claims, for example, of the hypotensive action of the Rauwolfia alkaloids as made on the basis of clinical studies could have been avoided by studies on the rat and dog.[21] A critical evaluation of the action of all potentially useful antihypertensive drugs on experimentally induced hypertension in animals would avoid the extravagant claims which have attended the introduction of the drugs now used as well as such agents as the nitrites, nitroglycerin, thiocyanate, etc., which were advocated for use during the earlier years of this century.

Most of the presently used drugs have been introduced on the basis of their pharmacologic action in normotensive, usually anesthetized animals. Maxwell[32] and his colleagues have recommended the pressor-blocking response as a screening procedure, an approach which led to their discovery of the hypotensive action of guanethidine. It must be remembered that one can modify the blood pressure by a variety of means, as, for example, by a reduction in cardiac output without a concomitant alteration in peripheral resistance, by a decrease in the blood and extracellular fluid volume without compensatory changes in vascular tone and cardiac output, by reducing the vascular tone without a corresponding increase in cardiac output, etc.[23]

The maintenance of the blood pressure at normal or abnormal levels involves a complex of neural autonomic reflexes, such as those subserved by the afferent endings in the carotid sinus and aortic arch and their efferent limbs in the vagus nerves and in the sympathetic trunks which innervate the heart, blood vessels, adrenal medulla, and other tissues. Humoral mechanisms, including the catecholamines as well as a variety of hormonal, central nervous system, renal, and other factors, participate in controlling the electrolyte-water metabolism, body fluid compartments, regional perfusion, and the other factors which contribute to blood pressure homeostasis. It is not surprising, therefore, that many drugs should affect the blood pressure in the normal as well as the hypertensive person and that

the deleterious effects of lowering the blood pressure may outweigh the beneficial influence of such action.

In assessment of the antihypertensive action of drugs in the clinic, one must always keep in mind the lability of the blood pressure and the ease with which nonspecific agents and placebos may appear to relieve symptoms and lower the blood pressure to some degree. The importance of the doctor-patient relationship, the powerful effect of a reassuring and enthusiastic approach to the patient, the effects of hospitalization, and other measures used to alleviate situational stresses necessitate the most critical approach, which unfortunately has not been followed in most published assays of antihypertensive drugs.[20]

Reserpine and other Rauwolfia alkaloids and their derivatives

There is a marked discrepancy between the pharmacologic effects of this group of drugs as studied on normotensive animals and their blood pressure–lowering effects as used clinically. In both the human and experimental hypertensive animal, their antihypertensive effects are unimpressive. Part of the discrepancy between the effects of the drugs in the laboratory and in the clinic is probably attributable to the fact that only a small fraction of the drug administered by mouth escapes destruction in the intestines. Only when administered parenterally, as is customary in treatment of patients suffering from malignant hypertension or hypertensive encephalopathy, is a good reduction in blood pressure attainable.

Pharmacology. The extensive pharmacologic studies of Bein,[5] Plummer and associates,[40] and others suggested that the lowering of the blood pressure and bradycardia induced by reserpine in animals are part of its general action in reducing predominance of sympathetic action as mediated by the hypothalamic centers. However, as shown by Carlsson and coauthors,[9] reserpine releases catecholamines from peripheral tissues as well as from the brain, and the cardiovascular responses to the action of the drug are due primarily to a loss of the transmitter at the peripheral nerve endings. Burn and Rand[8] have also demonstrated that reserpine depletes norepinephrine from the arterial walls. In small doses injected intravenously in animals, reserpine lowers the blood pressure and releases most of the peripheral norepinephrine without affecting measurably the norepinephrine or serotonin content of the brain.[35,37]

Reserpine acts by the release of norepinephrine as well as serotonin. The former action seems to be better correlated with its hypotensive action in animals than does its depressant effect on the vasomotor centers. In the human, on the other hand, a pronounced drop in blood pressure is usually observed in psychically disturbed or emotional persons whose blood pressure is excessively elevated because of psychic stimulation. Such patients, when in a more tranquil state regardless of how this may be induced, will manifest a decline in blood pressure. It is a failure to appreciate this elementary fact that is responsible for some of the early claims for the potent blood pressure–lowering effects attributed to the Rauwolfia compounds.[30]

Carefully controlled experiments have demonstrated that the Rauwolfia alkaloids, as used clinically, exert little antihypertensive action. Thus, in a double blind control study of antihypertensive agents by a group of the Veterans Administration on 326 male hypertensive patients followed up for at least 3 months with home blood pressure recordings, the effect of reserpine (0.5 mg. orally daily) alone in mild hypertension was statistically not significantly different than the results obtained after the administration of a placebo.[3]

In outpatient clinic patients with mild to moderately severe hypertension, Shapiro and Teng[45] also observed no difference between the effects on the systolic blood pressure of alseroxylon* (2 mg.), phenobarbi-

*Rauwiloid.

tal (30 mg.), or placebo, each administered three times a day. Slight but statistically significant differences were observed between the effects of the drugs and the placebo on the diastolic blood pressure (5 mm. in the case of phenobarbital, 10 mm. in the case of alseroxylon). Others have also shown that the decrease in blood pressure observed after the administration of reserpine alone is not important when subjected to critical analysis.[20,26]

The action of reserpine is complex, involving the intermediary role of serotonin centrally, the depletion of norepinephrine from both central and peripheral sites, with the release of catecholamines from the heart and other peripheral sites, and the stimulation of the activating reticular formation despite its sedative effects. However, as pointed out by Zimmerman and Sheppard,[46] the intensity of the gross pharmacologic effects of reserpine may not be correlated with the degree of change in the catecholamine and serotonin levels in the brain. These multiple actions would suggest the possibility of dissociating the hypotensive and sedative components of the action of reserpine by alterations in its molecular structure. Although deserpidine has been claimed to lack some of the undesirable side effects of reserpine, it and rescinnamine do not differ qualitatively from reserpine in pharmacologic effects.[53]

The carbethoxysyringoyl methyl ester of reserpine, syrosingopine, although retaining the hypotensive and bradycardiac actions of reserpine as tested in normotensive animals, is only mildly effective in eliminating the behavioral expression of fear and anxiety and in suppressing spontaneous activity in animals. The compound nevertheless retains the capacity of reserpine to antagonize morphine analgesia. It is claimed to exert one-tenth to one-fortieth the tranquilizing action of reserpine while retaining its hypotensive action.[28,40] The cardiac catechols are rapidly depleted by syrosingopine before the depletion of serotonin is marked. This would account for the dissociation of tranquilizing and hypotensive effects.[40,54]

Hydralazine

Hydralazine, 1-hydrazinophthalazine, has been demonstrated to exert an antihypertensive action in animals as well as in hypertensive patients.[21,26,30] Although originally thought to act centrally, it exerts peripheral effects; its mode of action, however, remains obscure. The drug apparently dampens sympathetic activity thereby reducing vascular tone. Schmid and Kellner[43] and others have demonstrated a decrease in peripheral resistance after administration of the drug, accompanied by an increased cardiac output and alterations in capillary and venous beds.

In animals, the drug exhibits sympatholytic properties.[29] It was originally claimed to be unique in lowering the blood pressure despite an increase in cardiac output and an increase in renal and cerebral blood flow. However, it is questionable if an increase in renal blood flow always occurs, since glomerular filtration is not increased. The increase in cardiac output may aggravate pre-existent coronary insufficiency or congestive heart failure or precipitate these.

Hydralazine increases the pulse pressure, pulse rate, and skin temperature, decreases the circulation time and peripheral resistance, and does not affect the blood volume. Its vasodilatory action is limited to relatively short vascular segments but occurs throughout the body.[29] These effects may result from a direct depressant action on the contractile elements of the blood vessels or from a reaction with receptors concerned in vasoconstriction. Although it exerts an adrenergic blocking action, hydralazine is neither specific, selective, nor potent in this respect.[29]

Clinical use. One would anticipate from its pharmacologic actions that hydralazine should exert potent antihypertensive activity in the human. Unfortunately, the large doses necessary for eliciting a good response, when the drug is used alone, are accompanied by such serious side effects as to preclude its use for this purpose. The lupuslike syndrome precipitated by the use

of the large doses advocated after the introduction of the drug may persist for long periods and has brought on a revulsion to the enthusiasm with which the drug was heralded originally. In moderate doses (not exceeding 150 to 200 mg. daily), however, the drug may be used effectively in combination with other agents, particularly the benzothiadiazine diuretics. In the cooperative study of the Veterans Administration,[3] hydralazine (200 mg. per day), in combination with reserpine, when used in moderately severe hypertension induced a mean change in diastolic pressure of −13.7 mm. Hg as compared to −4.3 mm. Hg with reserpine alone and 0.9 mm. Hg with placebos. This effect of reserpine plus hydralazine was as great as that obtained by the use of reserpine plus ganglion-blocking drugs.

Veratrum alkaloids

Mixtures of the veratrum preparations and their purified ester alkaloids, such as protoveratrines A and B and their mixture, reduce the blood pressure by stimulating the normal afferent reflex pathways from the baroreceptors in the heart and great vessels. This increased afferent stimulus to the vasomotor center results in bradycardia and a decreased efferent response with vasodilatation, particularly in the splanchnic bed. The depressor action of these drugs is a result of sensitization of the stretch receptors rather than of a central facilitation of the reflex arc.[42]

The erratic absorption of certain of the alkaloids from the intestines, the narrow margin between the dose required to lower the blood pressure and toxic symptoms (nausea, vomiting, substernal oppression, hiccough, extrasystoles, etc.), and the availability of other agents have deprived this group of their former popularity, so that they are now used infrequently except as components of mixtures of several antihypertensive drugs.

Despite their capacity to lower the blood pressure, the veratrum alkaloids suffer from the drawback that there is no dissociation of their hypotensive and emetic actions.[1] Protoveratrine A administered orally exerts a potent hypotensive action, but the dosage range between the desired and undesired effects is narrow and it exerts a cumulative action when administered at 6 hour intervals or less. Protoveratrine B, on the other hand, with variable amounts of which protoveratrine A is often marketed, is inactive orally in doses several times the hypotensive dose of protoveratrine A. The undesirability of utilizing such variable mixtures is obvious.[52]

Ganglion-blocking agents

A group of drugs which act by blocking the autonomic ganglia exert potent blood pressure–reducing activity and to a large extent have replaced the formerly popular surgical sympathectomy. As is to be anticipated, the very effective depressor action of these drugs and the fact that they indiscriminately inhibit parasympathetic as well as sympathetic impulses render their use fraught with undesirable side effects, so that they are used now only in the more advanced stages of hypertension.

Action. The ganglion-blocking agents lower the blood pressure by the reduction in vasomotor tone which ensues from paralysis of the peripheral sympathetic nerves. This results in a decrease in venous return and cardiac output without any appreciable decrease in peripheral resistance. Although these drugs are often believed to induce a chemical sympathectomy, this is only partially true. In animals it is difficult to achieve a complete sympathetic blockade by ganglioplegic drugs even in doses which would be intolerable in the human. As shown by Maxwell and colleagues,[33] the ganglion blockers even in high doses produce only a transient decline in diastolic pressure in the unanesthetized normotensive dog or rat or in the renal hypertensive or neurogenic hypertensive dog. Similar results are observed in the hypertensive rat.[21] Apparently, in the absence of anesthesia in the dog and rat (and presumably in man), such ganglioplegic drugs as

chlorisondamine and its congeners induce no pronounced block of the pathways which sustain the elevated diastolic pressure of the hypertensive patient.

The decline in blood pressure induced by the ganglioplegic drugs in the hypertensive patient is a consequence of the reduction in cardiac output, without change in the total peripheral resistance, which these drugs induce. The loss of vascular tone, as demonstrated by Smith and Hoobler,[47] involves the venomotor system, with only minor effects on arteriolar tone. As a result of this action, blood is shifted from the pulmonary to the peripheral circulation. The orthostatic hypotension which is the predominant clinical action of the ganglioplegic drugs results from partial blockade of the sympathetic nerves. This prevents the transmission of the normal impulses which counteract the decline in blood pressure induced by assuming the erect posture. Blockade of the sympathetic impulses to the arterial system by the ganglion blocking agents prevents the compensatory increase in peripheral arterial resistance which would normally occur in response to a decline in cardiac output.

Clinical use. The first of the ganglion-blocking agents used widely in the treatment of hypertension were the hexamethonium salts. The erratic absorption of this compound led to its replacement by a series of quaternary compounds which were reliable when administered orally and which proved to be effective in lowering the blood pressure but unfortunately are accompanied by often dangerous and intolerable side effects. These derivatives are now reserved for use only in the more seriously ill patients or those not controlled by other available measures.

In a double blind cooperative study at the Veterans Administration Hospitals, all three of the ganglion-blocking agents used—chlorisondamine, mecamylamine, and pentolinium tartrate—produced significant reductions of blood pressure (−16.5/−13.9 mm. Hg). The range of response was wide, varying from rises of blood pressure in approximately 20 per cent of patients to reductions of 40/30 mm. Hg in another 30 per cent. There was no significant difference in the antihypertensive effectiveness of the three drugs. Chlorisondamine was associated with more frequent disturbances of visual accommodation, whereas mecamylamine caused dryness of the mouth and difficult micturition more frequently than did the other drugs.[3]

Drugs that block sympathetic ganglia

The serious side effects that accompany blockade of the autonomic ganglia have led to a search for drugs which would limit their blocking action to the sympathetic without affecting the parasympathetic outflow. Two drugs have recently become available which aim to accomplish this purpose. Bretylium tosylate was introduced[6] into medical practice and used in Britain extensively in 1959 but has been subjected only to clinical trials in this country. The second drug, guanethidine sulfate,* has recently been marketed in the United States. The mechanism of action of the two drugs differs, but both lower the blood pressure by inhibiting peripheral sympathetic action.

Bretylium tosylate. Bretylium tosylate,† N-ortho-bromobenzyl-N-ethyl:N:N-dimethylammonium *p*-toluenesulfonate, selectively blocks peripheral adrenergic impulses without antagonizing the effects of injected epinephrine or norepinephrine.[13] It is thus antiadrenergic in action but not adrenolytic. Bretylium tosylate does not exert an appreciable action on parasympathetic function and thus is free of those undesirable side effects of the ganglion-blocking agents which are a consequence of parasympathetic blockade. The drug appears to act by inhibiting the release of neurohumoral mediators of the adrenergic nerves. Unlike guanethidine, it does not deplete the tissues of catecholamines but

*Ismelin.
†Darenthin.

interferes with the release of norepinephrine, the mediator of adrenergic impulses.

The principal side effects attending the use of bretylium tosylate include weakness, dizziness, ptosis of the eyelids, nasal stuffiness, etc. The severity of these symptoms does not correlate with the degree of lowering of the blood pressure, and tolerance to the drug develops with its continued use.[13]

Guanethidine. Guanethidine, [2-(octahydro-1-azocinyl)-ethyl]-guanidine sulfate, is unique structurally in having an eight-carbon ring. As demonstrated by Maxwell and his co-workers,[34] it acts on the sympathetic nerve endings without inhibiting parasympathetic action. Accordingly, it does not inhibit the bradycardiac effect of vagal stimulation but exhibits the typical effects of sympathetic inhibition as tested on the nictitating membrane of the eye. Guanethidine does not inhibit preganglionic or postganglionic transmission, neither does it act on the ganglionic synapses. Its action, accordingly, must be on the terminals of the sympathetic fibers, where it appears to act by interfering with the release or local transfer of the neurohumoral effector of the sympathetic nerve fibers.[34]

Guanethidine in doses of 7½ to 15 mg. per kilogram intravenously lowers markedly the arterial pressure of unanesthetized hypertensive dogs, with only slight effects on the blood pressure of the normotensive animal. In the anesthetized normotensive dog, it abolishes the pressor response of carotid occlusion and antagonizes the severe pressor responses elicited by high doses of amphetamine. The drug potentiates the effects of norepinephrine and to a lesser degree of epinephrine and is thus not adrenolytic in action.

Guanethidine is longer acting than bretylium tosylate and need be administered orally only once daily. The side effects attending use of the drug include diarrhea, dizziness, weakness, depression, failure of ejaculation, etc. Tolerance to the drug develops on continued use. Atropine may be added to control the diarrhea.[12,50]

Natriuretic drugs

This group of drugs now constitutes the most widely used of the antihypertensive agents.[50] Many utilize them as basic drug therapy, adding other drugs only when necessary in patients with the more severe forms of hypertension. This popularity of the natriuretic drugs is due not only to their effectiveness in lowering the blood pressure of many hypertensive patients but also to their relative freedom from the undesirable and dangerous side effects inherent in the use of other antihypertensive agents. The natriuretic agents also act synergistically with other measures and, for example, often reduce the blood pressure when this has not been affected by sympathectomy. When used in conjunction with ganglioplegic or sympatholytic drugs, the blood pressure may often be lowered effectively without the need of such large doses of the latter as induce intolerable side effects.[4,7,12,14,18,24]

Practically all of the newer nonmercurial diuretic drugs have been used in the treatment of hypertension.[50] Although the mercurial diuretics also exert a hypotensive action and were advocated for use in expediting the effect of sodium restriction in seriously ill patients, only the newer benzothiadiazine and phthalimidine derivatives have been used for routine treatment. These include chlorothiazide* and flumethiazide,† their hydro- derivatives‡ and dichlorhydro- derivatives,§ benzydroflumethiazide,|| and chlorthalidone.¶ Spironolactone,# an aldosterone-antagonizing agent, has received only preliminary trials. In view of the compensatory reaction of the adrenal cortex to the administration of this drug, its action would be anticipated to be transient except in the rare instances of primary hyperaldosteronism. Drugs inhibiting the production of aldosterone and

*Diuril.
†Ademol.
‡Hydrodiuril, Esidrix, Oretic, Saluron.
§Naqua.
||Naturetin.
¶Hygroton.
#Aldactone.

other pressor steroids by the adrenal cortex would offer better prospects as antihypertensive agents.

Mechanism of action. The preponderance of evidence indicates that the diuretic drugs lower the blood pressure in hypertension primarily as a consequence of their natriuretic action.[17,24] In this respect, therefore, they resemble the long-established efficacy of drastic sodium restriction; the results obtained by either drug or diet are comparable. Both decrease plasma and extracellular fluid volume, total exchangeable body sodium, and cardiac output and increase the responsiveness to other antihypertensive drugs and to sympathectomy.[2,10,15,17] The diuretics increase the total peripheral resistance. Although these effects accompany the lowering of the blood pressure after the initiation of drug or dietotherapy, subsequent adjustments in the organism occur with restoration of the plasma volume and cardiac output to pretreatment levels despite the maintenance of the decline in blood pressure. It is necessary to postulate, therefore, that long-term changes occur which counteract the basic disturbances which maintain the blood pressure at its elevated level.[10,12]

Deviations in the electrolyte and water balance, the volume of the body fluids, and the response to sodium depletion have been demonstrated to occur in hypertension, in both man and laboratory animals.[12,22,24,48,50] It is possible that a correction of these deviations is responsible for the long-term effects of the natriuretic drugs or sodium depletion in maintaining their hypotensive action and reducing the total peripheral vascular resistance.

In addition to the hemodynamic effects described above, the diuretic drugs, as well as sodium restriction, often depress glomerular filtration rate and cause a disproportionate increase in blood urea and an increase in the rate of reabsorption of free water.[11] These changes are noted after prolonged therapy; their significance in terms of the lowered blood pressure and the mechanism whereby they are induced can only be surmised.

Although the claim has been made that the benzothiadiazine derivatives exert their hypotensive action by mechanisms other than those concerned in the elimination of sodium, it is probable that such other effects play only a minor, if any, role, since equally striking effects are elicited in the human as well as in the experimental animal by simple sodium restriction.[12,24,50]

The fact that the diuretics are not hypotensive in normotensive individuals[51] affords no evidence to indicate that these drugs act as specific antihypertensive agents, for sodium restriction is also not hypotensive in the normal. The fact that the diuretics are more rapid and potent in action than is the dietary restriction of salt is also not unanticipated, since the latter procedure induces sodium depletion at a slower rate than the diuretic drugs. When due allowance is made for such time relationships, one finds in both animal and human experiments that a given depletion of sodium (without concomitant excessive losses of potassium) induces the same effect on the blood pressure regardless of whether this depletion be induced by dietary measures or by drug therapy.[12,50]

Clinical use. The availability of the potent, orally effective, relatively nontoxic natriuretic drugs has relegated sodium restriction by dietary means to a lesser importance in the management of hypertension. The difficulties inherent in dietary restriction as compared to the relative ease with which comparable degrees of sodium depletion can be obtained by drug therapy have resulted in the displacement of the former by the latter as the preferred therapeutic measure. Nevertheless, sodium restriction has the advantage over the use of natriuretic drugs of being free of such disturbances as potassium depletion, uric acid retention, and the rare sensitization phenomena which give rise to hematologic disturbances.[49,50]

In order to avoid such undesired side effects as accompany the use of natriuretic

drugs, it would appear logical to use these only as a supplement to moderate sodium restriction. The greater the degree of the latter, the lower the dose of the drug necessary for eliciting the desired decline in blood pressure. On the other hand, the drastic restriction of sodium required for eliciting a response and the use of onerous dietary restriction are no longer necessary.[12,41]

Drug combinations

A combination of several drugs and procedures is used frequently in the treatment of hypertension in an attempt to elicit maximum hypotensive action with a minimum of side effects. Numerous proprietary mixtures are available in which two or three drugs are combined. The rationale for such mixtures is questionable. In the case of sodium depletion by dietary restriction or by natriuretic drugs, it is well established that this regimen enhances the hypotensive effect of sympathectomy or the use of ganglioplegic or sympatholytic drugs.[50] The combined use of such drugs is therefore desirable, but since the optimal doses of each drug to be used in a given patient will vary, the advantage of fixed dosage combinations is questionable.

In the case of other combinations, such as hydralazine, veratrum, and the Rauwolfia alkaloids, etc., the evidence of a synergistic action when these are each used in a subeffective dose is not established. Since the mechanisms of action of the various drugs differ, one would anticipate that a combination of several of them would lower the blood pressure more effectively than any of the individual drugs used alone. However, there is no evidence to indicate that the use of one in subeffective doses would sensitize the vascular system to the hypotensive action of another drug and that the combination of drugs therefore would act synergistically. In any case, since the optimal dose for any given patient would vary, the use of fixed dosage combinations is irrational.

Role of drug therapy in hypertension

As already indicated, the antihypertensive agents in present use do not counteract the fundamental defect responsible for hypertensive disease; their action is comparable to that of the antipyretics in infectious fevers.[22] The question arises, therefore, as to their therapeutic value in lowering the blood pressure, which is only one manifestation of the disease.[23] In answering this question one must consider separately the various clinical stages of hypertensive disease.

In the "malignant" or accelerated stage of hypertensive disease, there is evidence to indicate that lowering the blood pressure by the presently available procedures (surgical operation, diet, or drugs) will in a fairly large proportion of patients induce a reversion of the disease to its more benign phase and prolong life.[27,31] It appears therefore that the inordinate elevation in arterial pressure per se contributes to the fatal disruptive vascular effects characteristic of this stage of the disease. Lowering of the pressure mitigates and restores the patient to a less serious form of the disease and is desirable if this can be accomplished without compromising renal, cardiac, or cerebral function. However, in patients with serious deficiency of function of the end organs primarily affected by the hypertensive process (heart, kidney, or brain), lowering of the blood pressure may have a deleterious and even fatal action.[20]

In the case of patients not suffering from the malignant phase of hypertension but from the less critical forms, the available evidence concerning the desirability and therapeutic value of lowering the blood pressure is less well established.[36,39]

In the case of milder forms of the disease, it is often possible to control the blood pressure by reassurance, the judicious use of phenobarbital, and diet.[38] Such simpler, safer, and cheaper measures would appear to be preferable to the use of the antihypertensive drugs, the use of which is attended by undesirable and often potentially dangerous side effects.

The common use of the term "hypertension" to designate the specific disease entity "hypertensive cardiovascular disease" as well as a group of unrelated conditions associated with an elevation in arterial blood pressure is unfortunate.[25] Such a lack of discrimination leads to emphasis on lowering the blood pressure, which may be undesirable and harmful in certain instances.[22] Thus, in the "systolic" hypertension of generalized arteriosclerosis, hemodynamic considerations would indicate the undesirability and potential harm inherent in lowering the elevated blood pressure.[23]

The rationale for utilizing drugs in lowering the blood pressure rests on the assumption that such lowering by its mechanical effects relieves the heart of its overwork and the blood vessels of overextension without in this process compromising the blood supply to the tissues. This cannot always be accomplished, particularly in the more advanced stages of the disease.

In the therapeutic use of antihypertensive drugs, one must balance the advantages to be gained by lowering the blood pressure against the undesired effects and dangers of such lowering. Such an empirical and symptomatic approach to the therapy of hypertension is unfortunately all that is available at present but should be replaced ultimately by more rational therapeutics in which the basic mechanism responsible for the disturbance is corrected. At present only rare cases of hypertensive disease are amenable to such an approach.

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In the long view, progress through research discloses itself as an evolutionary process. We may draw an analogy between the research performed consciously and with intent by man, and unconscious research on the part of nature. As man sets up experiments to find new truth, so does nature, in the case of living organisms at least, make experimental types through the process of mutation, and test them out in the struggle for existence. Thus we believe has the evolution of species come about, and in similar fashion has man acquired new knowledge and learned to improve his ways of approaching his objectives. As does nature, under an irresistible drive to procreate, force life to adapt itself to every environment, no matter how inimical, capable of supporting life at all, so does man under his drive to know, inquire into and explore every region of his cosmos to which his sensibilities and his intelligence direct him.

The clinical pharmacology of trace metals and iron

Rational use of a metal in the treatment of anemia depends upon an understanding of the mechanism by which the anemia develops and of the metabolism of that metal in relation to the formation of red blood cells. In order for a metal to be of value in the treatment of an anemia, there must be a deficiency of that metal and administration of that metal must correct the abnormality in red cell formation conditioned by the deficiency. The metabolism of the metals found in red cells and the circumstances under which a deficiency occurs are reviewed. Iron is the only metal for which there is any rational use in the treatment of anemia in man. The indications, contraindications, and methods of administering iron and the dangers of overdosage of iron are reviewed in the light of newer knowledge of the pathophysiology of iron.

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Red cells contain a number of metallic elements. Each participates in a metabolic cycle of its own in which it is absorbed and incorporated into developing red cells; it then usually remains in that cell to be released when the cell is destroyed. Upon release it may be excreted, stored, or reused for red cell formation. Incorporation of a metal into red cells is a complex process in which the following requirements must be met: (1) the metal must be present in the body in an amount adequate to satisfy the requirements for red cell production, (2) it must be made available at the site of red cell formation in a form suitable for incorporation into erythroblasts or reticulocytes, and (3)the erythroblast or reticulocyte must be capable of incorporating the metal into its stroma or into a specific substance containing the metal.

The rational use of a metal in the treatment of anemia in man necessitates an understanding of two interrelated phenomena: (1) the mechanism by which an anemia develops and (2) the metabolism of the metal in relation to the formation of red cells. Without this basic knowledge, treatment of anemia relapses into the empiricism reflected in the large number of hematinics manufactured by the drug industry. Most of these preparations contain agents which have no therapeutic value and are an unwarranted expense to the patient; others may be dangerous in certain circumstances.[21]

An anemia may be caused by a decreased rate of red cell production, an increased rate of red cell destruction, or

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hemorrhage or by a combination of two or all three of these factors. Hemorrhage occurs in two forms, into tissues and body cavities and from the body. In the former, the components of the red cell are preserved for reutilization in formation of new red cells; in the latter, none of the substances in the red cell are available for formation of red cells.

A metal is of use in the treatment of anemia only when (1) there is a deficiency of that metal, as a result of which the anemia develops, and (2) administration of the metal will correct the abnormality caused by the deficiency. For example, in the anemia of rheumatoid arthritis, all the constituents of the red cell are preserved for reutilization so that an anemia cannot be due to a deficiency of any component of the red cell. In Mediterranean anemia, the red cells are iron deficient because of a defect in incorporation of iron into hemoglobin and not a lack of iron. The anemia will therefore not respond to iron. In contrast, in chronic blood loss, all the components of the red cell including iron are lost. There is no defect in utilization of iron; therefore, iron is of benefit.

It is therefore the purpose of this article (1) to review the metabolism of trace metals and of iron in relation to the production of red cells and to the mechanism by which anemia develops and (2) to utilize this information to critically assess the role of trace metals and iron in the treatment of anemia in man.

From a therapeutic viewpoint, all metals known to be present in red cells may be classified into three groups: (1) those for which there is no known therapeutic use, (2) that whose therapeutic usefulness is still subject to debate, and (3) that which is known to be a useful therapeutic agent.

I. Metals with no known therapeutic use

Knowledge of the metabolism of the metals in this group is at best fragmentary. They are usually found in such small concentration in red cells, plasma, and tissues that the lack of suitable analytic techniques has been a major deterrent to further research. Newer techniques such as neutron activation may supply means to investigate these metals more adequately.[45]

A. Antimony. A comparatively high concentration of antimony in red cells is found within 8 days of intravenous injection of radioantimony as stibophen* or other antimony-containing drugs.[1] It is probable that antimony enters the red cells during the erythroblast stage since elements, such as iron, which are known to be incorporated into erythroblasts, also reach a comparatively high concentration in red cells at 8 days after intravenous administration.

Antimony deficiency does not exist in man, and there is no known therapeutic use for this metal in any abnormality of the red cell.

B. Copper. A great deal of information concerning the role of copper in erythropoiesis is now available primarily through the efforts of Cartwright and his associates.[14,15] In swine, a deficiency of copper induces an anemia that is strikingly similar to an iron-deficiency anemia. The copper deficiency causes decreased absorption and impaired utilization of iron, but unlike iron deficiency, there is also an increased rate of destruction of red cells. The anemia of copper deficiency responds to copper and not to iron.

Various interesting aspects of copper metabolism in the human infant and adult are now known.[14] At birth, there is an increased amount of tissue copper, presumably to compensate for the comparatively small amount of copper in human milk. Infants do not develop evidence of copper deficiency even when maintained on a copper-deficient diet for 4 to 5 months.

The daily dietary requirement of adult man is 2 mg. There is so much copper in the diet that it seems likely that a human would die of starvation prior to developing a deficiency of copper caused by a dietary insufficiency in this metal.

*Fuadin.

Abnormally large amounts of copper are excreted in nephrosis; however, administration of copper to patients with nephrosis has no effect upon the disease.[14]

In man, there is no condition known to be associated with or caused by copper deficiency and there is no rationale for the therapeutic use of copper.[14,48] Furthermore, there is no reason to believe that orally administered iron supplemented with copper is more effective in the treatment of iron deficiency than iron alone.

C. Magnesium. There is about 20 to 25 Gm. of magnesium in an average adult,[42,50] a comparatively large amount relative to other metals found in man. Most of this magnesium is located in bone and only trace amounts are found in red cells. Next to potassium, magnesium is the metal found in highest concentration in an intracellular location.[29]

Magnesium is known to play an important role in the metabolism of carbohydrates[23,29] and is needed for normal growth and development.[2,3] Abnormalities in magnesium metabolism are not known to have a direct effect upon erythropoiesis or upon the metabolism of the red cell.[3,12,29,37,48,50] There is therefore no rationale for the use of magnesium to treat abnormalities of the red cell in man.

D. Manganese. Clinical interest in manganese has centered about the resemblance of manganese toxicity, an industrial health problem, to Wilson's disease.[28] Excess amounts of manganese, apparently by blocking the action of or complexing with copper, induce a hypochromic microcytic anemia.

In rats, manganese deficiency leads to impaired growth and a loss of structural strength of bone. Under these circumstances, the rats also develop a mild anemia and a decreased capacity to recover from an anemia induced by phlebotomy.[49]

Radiomanganese, after intravenous injection in animals, is rapidly concentrated in cells (liver, kidney, and pancreas) rich in mitochondria. However, within 36 to 48 hours, a major portion of the intravenously injected radiomanganese is found in red cells.[18]

In adult man, the total body manganese is approximately 20 mg. There is no rationale for the use of manganese in any disease affecting red cells.[17,48]

E. Molybdenum. Practically nothing is known about the role played by molybdenum in man. In cattle, increased absorption of molybdenum induces a condition similar to copper deficiency.[14]

In recent years, combinations of ferrous sulfate and molybdenum oxide (in some preparations vitamins and calcium have also been added) have been marketed for the treatment of iron deficiency. There is no reason to believe that a combination of iron plus molybdenum (or plus the other substances mentioned) has any advantage over iron alone in the treatment of patients with iron deficiency.

F. Zinc. Zinc is the most intensively studied of all trace metals found in red cells. In man, there is approximately 12 to 14 μg of zinc per 1 ml. of red cells.[4,44,46] The concentration of red cell zinc parallels that of carbonic anhydrase.[4,46,47] The concentration of both in red cells remains unchanged in spite of wide variations in the number of red cells in a variety of diseases.[47] However, pernicious anemia is a notable exception in that in relapse the red cell concentration of both zinc and carbonic anhydrase is higher than normal. This increased concentration returns to normal after treatment of the pernicious anemia, coinciding with the replacement of the abnormal red cells of pernicious anemia by normal red cells. More recently Talbott and Ross[44] demonstrated an increased concentration of zinc in the red cells of patients with myeloid metaplasia and reticulum cell sarcoma. The significance of these alterations in concentration of zinc in red cells is unknown.

According to Berfenstam,[4] the average diet contains 10 to 12 mg. of zinc per day. Feeding zinc to pregnant women does not change the concentration of zinc in the red cells of newborn infants. Furthermore, there

is more zinc in the food than can be absorbed by even a rapidly growing infant. These facts indicate that a dietary deficiency of zinc is extremely unlikely to develop in man.[4] There is no rationale for the use of zinc in treatment of diseases affecting red cells in man.

II. A metal of doubtful therapeutic value

Cobalt, the only metal in this category, has been suspected of playing a key role in erythropoiesis because cobalt chloride induces a polycythemia in some laboratory mammals[39] and because cobalt is part of the B_{12} molecule. The mechanism by which this polycythemia develops in unknown.

Cobalt given in doses of 80 to 200 mg. daily for several weeks will induce a 10 to 20 per cent rise in hemoglobin of patients whose anemia is secondary to azotemia.[25] This dose will also cause nausea and vomiting,[25] a most undesirable complication in an azotemic patient. A similar response in hemoglobin and the same incidence of toxic reactions follow the use of cobalt in the treatment of anemia caused by infection[5,14] and cancer.[14,40] The mechanism by which cobalt induces a rise in hemoglobin in these diseases is unknown. Hypothyroidism with hyperplasia of the thyroid gland is another undesirable side effect ascribed to treatment with cobalt.[14]

The use of cobalt in the treatment of various anemias in man was the subject of a symposium in a 1955 issue of *Blood*.[19] There was agreement among the fourteen panelists with regard to the following points: (1) cobalt in doses over 100 mg. daily will induce a rise in hemoglobin in nephritis, cancer, and chronic infections; (2) this rise is not of clinical significance; (3) the toxic effects at doses needed to produce a rise in hemoglobin are serious enough to make its use inadvisable; and (4) cobalt chloride used with ferrous sulfate has no advantage over ferrous sulfate alone in the treatment of iron deficiency anemia. The moderator concluded that "because of the almost complete unanimity of the panel in this regard and because of the possible toxicity of cobalt in some patients, it seems unwise to recommend either cobalt alone or cobalt with iron to the medical profession."

III. Iron—The metal of established therapeutic value

Knowledge of the metabolism of iron is so advanced that it is possible to determine who will benefit from treatment with iron and who will not and to prescribe iron in the cheapest and most efficient manner. Nevertheless, the *Physicians' Desk Reference*,[36] prepared with the cooperation of the "ethical" drug companies, lists such a wide variety of iron preparations, with so many added substances of no conceivable use, that it is obvious that the information now available on iron metabolism has not reached the majority of the medical profession. Yet, a knowledge of the basic aspects of iron metabolism is absolutely essential for the rational use of iron.

A. Iron metabolism.

1. Total body iron. In an adult man, approximately 2,400 mg. of iron (1 mg. per 1 ml. of red cells) is stored in the hemoglobin of red cells and an additional 1,200 mg. is stored as ferritin in hematopoietic tissues and hepatic parenchymal cells.[26] Ferritin is a complex of iron and the protein apoferritin in which 23 per cent of the molecule is iron. When iron stores are larger than normal, iron is deposited as hemosiderin, also a complex of iron and apoferritin, 35 per cent of whose weight is iron. In all other respects, ferritin and hemosiderin are essentially similar.[11] The small amounts of iron in myoglobin and in enzymes are of little if any significance in the pathophysiology of anemia.

The total body iron is governed solely by the amount lost and the amount absorbed. Men lose a total of about 1 mg. daily in bile, urine, and sweat and in the exfoliation of cells from the body.[20] In women, the menstrual flow results in an additional 20 to 30 mg. loss (corresponding

approximately to the blood absorbed by 12 menstrual pads). The menstruating female over the course of each 30 days therefore has an iron loss approximately twice that of the average man. In an uncomplicated pregnancy, there is a loss of about 400 mg. of iron to the fetus, 150 mg. in the postpartum flow, and 200 mg. in the placenta. During pregnancy, 270 mg. of iron is conserved because of amenorrhea. Therefore, an average pregnancy represents a net loss of 480 mg. over the nongravid state. *There is no mechanism to increase loss of iron except by hemorrhage from the body.*

The regulation of absorption of iron is poorly understood. The simple explanation that this absorption depends upon the ratio of apoferritin to ferritin in the gastrointestinal tract is no longer tenable.[11] Normally, the absorption of iron is maintained approximately equal to iron loss.

Based on a hemoglobin level of 15 Gm. per 100 ml., a red cell volume of 2,400 ml., and tissue stores of 1,200 mg. of iron, it would take 1,200 days or about 3 years on an absolutely iron-free diet before the average adult male, in the absence of hemorrhage from the body, could lose enough iron to develop a detectable anemia and another 3 years before the hemoglobin content of the blood would fall to 7.5 Gm. per 100 ml. In a menstruating female on a similar diet, this would occur about 20 per cent more rapidly because of a smaller blood volume, smaller tissue stores of iron, and a greater blood loss because of the menstrual flow. These calculations represent an inconceivable situation since an iron-free diet is almost an impossibility and since, according to Moore,[34] absorption of iron from food increases with the severity of iron deficiency. A clinically significant decrease in total body iron (iron deficiency) solely as a result of decreased absorption of iron is therefore comparatively rare and very slow to develop.

In contrast, the amount of body iron may easily be increased by an increased absorption of iron.[11] Anemic patients[35] and animals,[32] regardless of whether or not they are iron deficient, will absorb a larger than normal amount of dietary iron. The continued ingestion of a diet containing excessive amounts of iron will result in the absorption of abnormally large amounts of iron.[30,31,51] Injection of parenteral iron or transfusion of blood is the most rapid and most effective means to increase the total body iron. Each transfusion of 500 ml. of whole blood contains approximately 200 mg. of iron. However, the use of blood transfusion as a means of administration of iron is absolutely unwarranted.

These observations lead to the following conclusions applicable to adults: (1) a decrease in total body iron (iron deficiency) is almost invariably caused by hemorrhage from the body and (2) a decrease in body iron, even if known to be associated with decreased absorption of iron, is almost always aggravated by an accompanying, often occult, loss of blood.

The growing child represents a special circumstance. Additional iron must be absorbed to provide for the increase in body mass and in red cell volume. According to Moore,[34] a growing male or female must accumulate approximately 0.6 mg. of iron daily between birth and 20 years of age. Increased absorption of dietary iron is the only physiologic source for this increment of 0.6 mg. of iron daily. Therefore, a defect in absorption of iron is more frequently a contributing cause of decreased iron stores in the growing child than in an adult.[41]

2. *Distribution and utilization of iron.* Anemia not caused by hemorrhage from the body must result from either a decrease in the production or an increase in destruction of red cells or from a combination of both factors. In any event, the rate of incorporation of iron into the hemoglobin of newly formed red cells will be less than the rate of release from hemoglobin from destruction of senescent red cells, leading to a decrease in the amount of red cell iron. The iron no longer contained in red cells must shift to the tissues for the following reasons: (1) the plasma can contain only an

additional 6 mg. of iron,[33] (2) the iron cannot be excreted, and (3) the iron cannot be incorporated into myoglobin or into enzymes containing iron. Therefore, in an anemia not resulting from blood loss from the body, there can only be a redistribution of iron and not an iron deficiency.[53] Treatment of such a patient with iron is useless since the additional iron will not increase the rate of red cell production or decrease the rate of red cell destruction.

The iron administered under these conditions will be quantitatively transferred to tissue stores as hemosiderin or ferritin.[27,52,53]

On the other hand, an entirely different sequence of events occurs when the anemia is caused by hemorrhage from the body. If the patient is not debilitated by serious illness the fall in red cell volume causes a compensatory increase in red cell production, with an increased rate of incorporation of iron into the hemoglobin of newly forming red cells.[16,22] This increased requirement for iron is not met by the release of iron from hemoglobin from the destruction of senescent red cells because there is no increase in the destruction of red cells. There is only an inadequate increase in absorption of iron from food. Therefore, most of the increased amount of iron needed for incorporation into the hemoglobin of newly formed red cells must come from tissue stores. Eventually, with continued blood loss, these tissue stores will be depleted of iron. Thereafter, the only iron available for hemoglobin synthesis will be the inadequate amounts released from destruction of senescent red cells and iron absorbed from food. The rate of red cell formation will fall because of a lack of iron for hemoglobin synthesis.[22] Iron therapy, by correcting the iron deficit, will then increase the rate of red cell formation until a normal red cell volume and hemoglobin are reconstituted.

B. Iron deficiency. By rigid definition, an iron deficiency exists whenever the total body iron is less than the norm for that individual. The outmoded concept that iron deficiency is found only in patients who are anemic or who have red cells with the morphologic characteristics of iron-deficient red cells, or Wintrobe indices characteristic of iron deficiency, or a low serum iron and elevated iron-binding capacity must be disregarded.[6-8,10] A patient may be considered iron deficient, even in the absence of red cell or plasma abnormalities, if it can be demonstrated by biopsy that there is a deficit in tissue iron or if there is an unequivocal past history of blood loss from the body. This type of iron deficiency is usually not accompanied by any symptoms other than brittleness and decrease in the rate of nail growth.[10]

C. Rational use of iron. There is only one clinical use for iron—treatment of the iron-deficient patient. However, not all iron-deficient patients are able to respond to iron. In order to introduce iron into hemoglobin in red cells, the patient with an iron-deficiency anemia must increase his rate of formation of red cells and hemoglobin. If the iron deficiency is accompanied by a serious intercurrent illness, the iron-deficient patient cannot increase his rate of red cell and hemoglobin formation[24,52] and so will not respond to iron until the intercurrent disease has been treated or its toxic manifestations alleviated.

The response to therapy cannot be judged solely on the basis of a rise in red cell count, hemoglobin, or hematocrit if the blood loss which was the cause of the iron deficiency has not been corrected. Provided adequate amounts of iron are supplied, an iron-deficient patient without serious intercurrent debilitating disease is able to form about 60 ml. of red cells daily.[16] Reticulocytosis may, therefore, be the only evidence that the patient is responding to iron therapy if he continues to lose more than 60 ml. of blood per day (barely enough to make the stools tarry when bleeding is gastrointestinal in origin).

The most common error in treatment of the iron-deficient patient is the failure of the physician to search for the source of hemorrhage which is almost invariably present.

D. Selection of an iron preparation. Ideally, a preparation used for the treatment of iron deficiency should have the following characteristics: (1) the iron should be completely absorbed from the gastrointestinal tract, (2) the tissue iron as well as the hemoglobin mass should be reconstituted by it, (3) the compound should not contain material effective in treatment of any anemia other than that caused by iron deficiency, (4) it should not be toxic, (5) it should not cause gastrointestinal irritation, and (6) it should not be expensive.

Ferrous sulfate, in pill or tablet form for adults and in solution for children, is a compound which meets these criteria. The effectiveness of ferrous sulfate is not increased by the addition of trace metals or vitamins.[9] The increment in absorption of iron after addition of vitamin C is of no clinical significance. Adding hydrochloride will not significantly increase the absorption of iron but will erode the enamel of the teeth.

Ferrous sulfate has only a few disadvantages. About 10 to 20 per cent of patients develop gastrointestinal symptoms of such severity as to preclude its use.[43] This complication may be avoided by giving the ferrous sulfate immediately after meals. Other ferrous salts, such as the gluconate, may be better tolerated, but some patients are not able to tolerate any oral iron preparation and must be treated with parenteral iron. Another disadvantage is that ferrous sulfate (as well as all other oral compounds) must be given continuously for several months after the hemoglobin has been reconstituted before tissue stores of iron have returned to normal.[6]

Theoretically, enough iron must be given to rebuild the hemoglobin of red cells and the tissue iron to a normal level. A rise of 2 Gm. per 100 ml. or of 5 per cent in hematocrit within 3 weeks is usually accepted as evidence of a satisfactory rate of red cell regeneration.[43] This corresponds roughly to the daily synthesis of 40 to 60 mg. of iron into the hemoglobin of newly formed red cells. Ideally, by the time the hemoglobin has returned to normal, enough additional iron should have been absorbed to provide for incorporation of 1,200 mg. of iron into ferritin. From a practical viewpoint, it is much simpler to prescribe 0.6 Gm. of ferrous sulfate three times daily for about 3 to 6 months after the hemoglobin has returned to normal. A more accurate calculation of the oral dose is impossible because the absorption of iron decreases as the iron deficiency is alleviated by treatment.[34]

The milder type of iron deficiency in which there is solely depletion of tissue iron or only a minor degree of anemia may be treated by 0.6 Gm. of ferrous sulfate for 3 to 6 months since, as already mentioned, a more accurate calculation of an oral dose is impossible. A more expeditious and practical method is parenteral injection of 1,200 mg. of iron.

The earliest preparations of iron for intravenous use were followed by a high incidence of reactions. However, with the development of chelates of iron, especially saccharated iron oxide, the great majority of these reactions have been eliminated. The true incidence of reactions to intravenously administered iron dextran is unknown. In my own experience, approximately 1 per cent of patients will develop chills, fever, or urticaria. Neurocirculatory collapse and even fatalities have been reported. If a maximum of 100 to 200 mg. of iron is injected slowly at one time, the incidence of reactions is lessened.

Other major disadvantages of intravenous iron are the inconvenience of intravenous therapy, especially in children and women, and the comparatively high cost of intravenous iron.

The advantages of intravenous administration of iron are as follows: Gastrointestinal symptoms do not occur. Patients with a malabsorption syndrome, such as associated with sprue, may be given adequate amounts of iron. By injection of larger amounts of iron than the 60 mg. that can be utilized daily in red cell formation, the tissue stores may be rebuilt even before

the hemoglobin has returned to normal. It is possible to build up excess stores of iron from which a patient may draw to maintain a normal hemoglobin in the face of anticipated continued hemorrhage. Parenteral iron may be the only way to be certain an unreliable patient is actually being treated with adequate amounts of iron.

Many of the disadvantages of intravenous iron were obviated and the advantages retained with the introduction of iron dextran for intramuscular use. The incidence of allergic reactions remained unchanged, but the dangerous toxic reactions such as vascular collapse seemed decreased in number. Iron dextran given intramuscularly gained such popularity that, according to the manufacturers, several million injections have been given, a number far in excess of any conceivable rational use of iron.

The compound has been voluntarily retired from the market by the manufacturers (and is no longer available) because of reports of the induction of sarcomas at the site of intramuscular injection in rats.[38] In view of the huge dose of iron dextran given to these animals and in view of the known tendency of this animal to develop sarcomas, there is some question whether the results are applicable to man. The much more important problem, the treatment by iron of large numbers of patients who do not have an iron deficiency, has been neglected.

Some investigators have considered the possibility of producing hemochromatosis in patients given an overdose of iron, especially when parenteral iron has been administered.[43] This possibility is now considered to be remote for the following reasons. The accumulation of excess stores of iron will not in itself lead to hemochromatosis in man[11] or animals.[13] In order to develop hemochromatosis, two abnormalities must coexist: (1) increased iron stores and (2) an abnormality in the way in which the iron is handled by the patient.

The dosage of intravenous or intramuscular iron may be calculated provided one remembers that the purpose of iron medication is to reconstitute the hemoglobin and tissue stores of iron *and* that the tissue iron is always utilized for hemoglobin formation before the patient becomes anemic when the anemia is due to chronic blood loss. A typical situation is a male with a level of 7.5 Gm. per 100 ml. hemoglobin resulting from chronic blood loss from a duodenal ulcer. The normal hemoglobin is considered to be 15 Gm. per 100 ml. and the normal red cell volume 2,400 ml. These red cells contain 2,400 mg. of iron. Since the hemoglobin is half of normal, the red cells can contain only half the normal amount of iron. Also, since tissue iron was depleted prior to the development of anemia, there must in addition be a tissue deficit of 1,200 mg. Regardless of the degree of anemia in chronic blood loss, 1,200 mg. will be needed to replace the tissue iron. The total dose of iron needed, provided of course that bleeding does not continue, is therefore 2,400 mg. of iron (1,200 mg. to reconstitute the hemoglobin and 1,200 mg. to reconstitute the tissue stores). If the bleeding is acute, a dose of iron adequate to reconstitute the hemoglobin loss is administered.

This calculation does not take into consideration iron that will be lost if the patient continues to bleed. The iron lost in this way may be replaced by injecting an additional amount of iron equal in milligrams to the patient's hematocrit multiplied by the volume of blood lost. For example, 16 mg. of iron should be injected for each 80 ml. of whole blood when the hematocrit is 20 per cent. An overdose of a few grams of iron is not harmful.[11] If the patient continues to bleed, this excess iron will eventually be used for hemoglobin synthesis.

Iron may be injected intramuscularly in doses of 100 to 200 mg. daily or every other day. More rapid treatment or the use of larger doses will not induce any more rapid formation of hemoglobin. The severity and frequency of local and systemic reactions will increase if the size of dose or frequency of injection is exceeded.

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Hypothermia—Physiologic rationale

Hypothermia is useful in elective cardiac surgery, neurosurgery, general surgery, and internal medicine. This broad application requires an understanding of the functional effects over a wider range of temperatures to permit its intelligent clinical application. Except in the range 34° to 32° C., the magnitude of physiologic depressive changes is roughly proportional to the depth of cooling. This range (the "augmented" level), while reducing metabolism about one-third, produces a vascular pressor response, a slowing pulse, and hyperreflexia and enhances ventilation.

At 30° C. (moderate), the first serious functional changes develop. Blood flow is 50 per cent of normal, as is oxygen utilization. Ventilation becomes depressed. Arterial blood pressure is maintained. Myocardial efficiency is at its peak. At 25° C. (moderately deep), hypotension develops with more marked bradycardia. Blood flow is less than one-third of normal. Electrocardiogram abnormalities become more prominently manifest. Cardiovascular reflexes are depressed. At 20° C. (deep), central control of homeostasis is depressed, and below 15° C. (severe), signs of suspended animation such as cold cardiac arrest appear. Duration of permissible cooling is inversely proportional to the depth of cooling. Patients have been kept at 32° C. for 3 weeks with normal resuscitation. At the deeper levels, more rigid control is required. For the present, these levels can be maintained for short periods only and require rapid cooling and rewarming.

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Clinical use of hypothermia falls into several broad categories: (1) cardiovascular surgery, (2) neurosurgery, (3) general surgery, and (4) general medicine, including infectious diseases. This division of application is not merely a matter of clinical discipline but most specifically relates to the manner, depth, degree, and duration of cooling employed. The determination of the above and, of course, the initial decision to use hypothermia at all are based upon the recognized physiologic changes induced. Therapeutic hypothermia is not merely cooling a patient because of presumed benefit but involves an assessment of its value with particular reference to depth, hence, functional alterations. This report constitutes a brief review of some of the effects of hypothermia on the physiologic status of the homeother-

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mic being, with special consideration of the desirable features for possible general therapeutic use.

Generally, hypothermia is considered a part of the disciplines of cardiac surgery and, more recently, neurosurgery. Actually, this use of cold is only very recent. Attempts at utilization of human cooling date back at least 150 years. In 1772 Robert Boyle suggested the beneficial uses of cold.[27] James Currie[14] in 1797 published his successful clinical experiences in the treatment of high fevers.

Unheralded abortive attempts at reintroducing cold smoldered until the 1940s. Smith and Fay[40] reported success in arresting pain in cancer and presumably its growth. Bigelow,[5] Lewis, Ring, and Alden,[24] and Talbott[44] recommended cooling poor risk, extremely ill patients. It has proved a valuable adjunct in the treatment of septic shock.[1] Cardiac surgery rapidly matured as the potential of hypothermia was developed and soon overshadowed other applications.

Equally as important as these clinical experiences is the fact that hypothermia has long been an established research tool. Currie[14] recorded subnormal temperatures in normal subjects immersed in brine. While his observations were scanty by modern standards, he did establish elective cooling of the human, although this was soon forgotten. Frequent observations of accidentally cooled people (as low as 24° C. with survival) have been reported since the midnineteenth century.[16,36] Markwalder and Starling[28] in 1914 recorded the effects of cold on cardiac output in the heart-lung preparation. Cannon and associates[13] in 1927 studied the effects of local gastric cooling, which procedure has been used with great success by Wangensteen and his group.[46] In recent years, tremendous effort has been exercised in exploring the effects of cold. Much controversy has resulted, but at the same time a great deal has been learned. It is possible now to achieve an adequate appraisal of the effects of various depths of hypothermia and establish a rationale for its use under a variety of circumstances.

As a matter of definition, hypothermia is low body temperature in a homothermic being. It is not artificial hibernation, as it is so often popularly called. Natural hibernators can be made to hibernate artificially, this status being relatively normal for them. Homeothermic beings, on the other hand, when rendered below normal temperature, are in an abnormal and potentially risky state.

Physiology

In the consideration of functional effects of hypothermia, a number of modifying factors must be kept in mind. Difference in species of animals used in experiments, anesthetic effects on homeostatic mechanisms, and duration of the hypothermic state contribute high degrees of variance to research findings. Effects of drugs on temperature-regulating mechanisms are of obvious importance, and here a depressant effect often is instituted deliberately. The method of cooling exerts a significant modification, depending upon the rate and the degree of cooling of the superficial tissues in relation to the deep core. In general, two techniques for elective temperature alteration are available: surface contact and via the blood stream. The former is used for more moderate and prolonged periods of clinical cooling (33° to 28° C.). Ice water bath immersion, ice bags, cold air circulation, and refrigeration blankets have been utilized, the last being the most popular. Large temperature gradients develop with these techniques, and considerable drift in temperature occurs ("after-fall" on cooling and "overshoot" on rewarming) after removal from the cold or hot environment.[42] The drift is thought to be due to attempted equilibration between superficial and deep core temperatures toward the normal gradient of about 2° C. Unless the "after-fall" is controlled, the temperature may drift beyond the desired level to those at which greater functional depression occurs. Upon rewarming, the superfi-

cial vessels dilate, pooling blood in the peripheries and increasing surface tissue metabolic demands, while the heart is still cold and moving blood at the correspondingly slow rate. A shocklike state may develop.[9] Metabolic acidosis may develop.[45] An approach in the management of this problem is to allow the patient to rewarm at his own rate.

Blood stream cooling and rewarming is more rapid, efficient, and controllable. However, disadvantages lie in the complexity of equipment, intravascular cannulation with potential hazards of thrombosis and infection, and the need for constant supervision. Use of this technique for the present has been limited to intracardiac surgery, wherein the period of hypothermia is maintained for an hour or less.

Effect of depth is one of progressive depression of function, as would be expected. Of equal importance is the duration of cooling, as related to depth. The longer a subject is kept at a deep level (below 30° C.), the greater the risk of irreversible changes or even death. Observations in humans have not been made under ideal experimental conditions for obvious reasons. Primary fundamental problems, such as immunologic responses, have not yet been touched. Despite these shortcomings, however, adequate data are available not only for analysis but also for the enhancement of a logical approach to the clinical use of hypothermia.

The levels of cooling (33° C., 30° C., 25° C., and 20° C.) selected for discussion in this report are those (1) which have been found to be therapeutically most useful and (2) in which significant physiologic alterations begin to appear.

Metabolism, respiration, and ventilation. The fundamental tenet in the use of hypothermia lies in the reduction of metabolism, as measured by oxygen consumption. The magnitude of change is generally linear with temperature fall.[41] Clinically significant changes are shown in Fig. 1. At 33° C., oxygen utilization is but two-thirds of normal and only one-half at 30° C. Below 20° C., the decrease reaches a point where utilization is considered almost minimal. Below 15° C., the requirement is so low that perfusion can be stopped for significant periods of time. However, there does still exist some oxygen requirement albeit small, as pointed out by Gollan.[20] In the conscious human, in whom shivering occurs, oxygen consumption is increased.[17] This results from heightened striated muscle activity in response to the demand for increased heat production. These studies serve to point out the need for abolishing this shivering. With adequate control of shivering through anesthetics and curarizing agents, oxygen use falls exponentially as in experimental animals.[26] Oxygen consumption, as an index of metabolism, certainly is not the entire picture. Anaerobic metabolism may account for some of the observed adequacy of function during hypothermia as availability of oxygen is reduced. However, this has not been established.[47] The reduction in oxygen requirement, in any event, provides the essential key to the assessment of the value of hypothermia and the level to be used. This reduction can occur only in

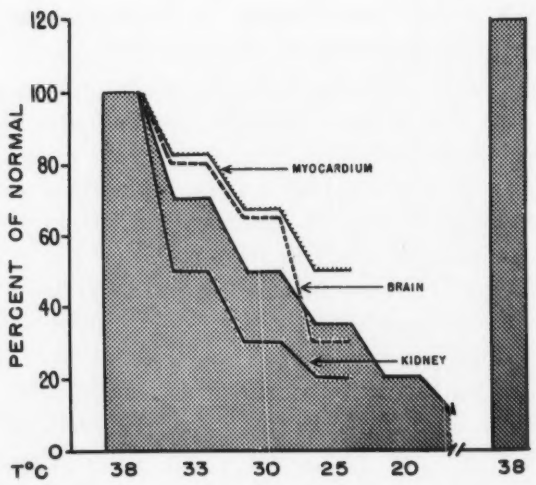

Fig. 1. Oxygen consumption with hypothermia. The magnitude of reduction of oxygen requirement of brain, myocardium, and kidney in relation to over-all utilization is demonstrated. Renal requirement shows the greatest reduction for each temperature level.

Table I. *Ventilation with hypothermia* (median range)*

Temperature (C.)	*Rate per minute*	*Ventilation (L. per min.)*	*Helium clearance (min.)*	*Functional residual capacity (ml.)*	*Ventilation efficiency ratio* $\left(\frac{mv}{He_t}\right)$
37	20–30	2–3	1–2	200–400	Below 0.2†
33	—	1.5	2–3	200–400	Below 0.2
30	5–8	0.5–1.5	3–6	250–300	0.3–0.5
25	5	Below 0.5	6–7	200–250	0.7–2.0
37 (Rewarm)	15–20	2–3	1–2	300–500	Below 0.2

*Chlorolosed dogs.
†Upper limit of normal.

diminished heat production, both shivering and nonshivering (nonskeletal). Increased striated muscle activity is the shivering part of heat production. Nonshivering sources of thermogenesis are not as well established and are more complex.[15] They are believed to involve primarily central temperature change and also humoral, biochemical, and enzymatic systems. Control of heat production is aimed primarily at the shivering source either by anesthesia or such agents as phenothiazine derivatives. Resistance to cooling may be absent in the seriously ill patient, due to depression of temperature reflex mechanisms.*

Blood cooled in vitro becomes more alkaline.[32] Shift in the oxygen dissociation curve, again in vitro, is well known. However, these alterations for in vivo blood are not as simple, because of respiratory and renal functional effects, although Severinghaus, Stupfel, and Bradley[38] feel cold in vivo blood manifests similar changes. At deeper levels of hypothermia (25° C. and below), these physicochemical differences could be of potential harm. Some amelioration might be expected in the form of respiratory acidosis with lowering of blood pH which is often seen at the deep levels.[2] In acidosis the oxygen dissociation shift is to the right and thus would counterbalance the shift caused by cold. It appears that despite potential impairment of oxygen transport, hypoxia during hypothermia is not seen.

Low alveolar carbon dioxide partial pressure with increased arterial blood carbon dioxide content has been observed during cold.[17] This apparent discrepancy is felt to be due to the greater solubility of carbon dioxide at low temperatures and could account for the increased content in vitro and in vivo. The decreased elimination of carbon dioxide because of greater solubility in plasma and of lowered partial pressure is balanced by the lesser production which follows the decreased metabolism during hypothermia.[47] Along with the increased solubility of carbon dioxide (25° C.), the dissociation of carbonic acid diminishes and cation from protein enters the bicarbonate–carbonic acid buffer system more readily.[2]

Of prime significance in this matter of gas transport and carbon dioxide elimination is the *depth* of the cold. The changes are reported at 25° C. and below. At the more moderate levels recommended for general therapy (above 30° C.), it is likely these alterations do not occur to a significant degree, and further, compensatory respiratory and renal mechanisms are not seriously deterred.

Diffusion (carbon monoxide equilibration technique) is normal to 25° C., the lowest studied. The dead space appears to be increased somewhat again only around 25° C.[42] Ventilation is augmented around

*E. Blair: Unpublished data.

Table II. *Hemodynamics with hypothermia**

Data	*Temperature (C.)*				
	37°	*33°*	*30°*	*26°*	*34°*
Mean arterial blood pressure (mm. Hg)	78	96	80	64	82
Pulse pressure (mm. Hg)	38	40	40	20	46
Heart rate (min.)	118	96	60	40	90

*Mean values of clinical data.

33° C., especially in ill patients.* Depression of breathing, however, is manifest at the moderate level of 30° C.[6] (Table I). At 25° C., the minute ventilation averages 0.5 L. compared to a median range of 2 to 3 L. at 37° C. On rewarming, ventilation returns to normal. Of special note is the development of significant maldistribution of air. As measured by helium clearance techniques, the rate of washout is three and five times longer at 30° and 25° C., respectively, compared to normal. The functional residual (mid) capacity shows considerable variation and no consistent alteration except at 25° C., at which it is smaller. On rewarming, all of these activities return to normal. The ventilation efficiency ratio, an index of over-all ventilation, indicates adequate ventilation at 32° C., reduced efficiency beginning at 30° C., and marked incapacity at 25° C., with return to normal on rewarming. These observations were made in experimental animals. Changes in the human have not been well defined. The depression during hypothermia is of central and metabolic origins, the lung itself apparently manifesting no unusual change. Evidence for the former lies in diminished respiratory response to carbon dioxide inhalation (below 25° C.).[12]

Cardiovascular. A significant consequence of the reduced oxygen requirement during hypothermia is the reduced need for perfusion. This means that the heart will not have to work as much, a most beneficial gesture to a diseased myocardium. Table II shows clinical data derived from patients undergoing cardiovascular surgical operations. The pulse slows. Below 15° C., cold arrest ensues. Another singular effect of cooling is the pressor effect of cold which occurs in both humans and in experimental animals.[7] This takes place between 35 and 32° C. and is manifested by a rise in arterial blood pressure. This has been a consistent observation in cooling patients in shock, especially *nonhemorrhagic.* It is likely a direct pressor effect of cold.[3,39] At 30° C., the blood pressure is within normal limits, and only below this level does hypotension ensue. Peripheral venous pressure rises somewhat during cooling. Serious elevations in central venous pressure may be seen in deep hypothermia over prolonged periods.[8] The A-V oxygen difference remains normal through 20° C. On rewarming after prolonged hypothermia, an increase in A-V oxygen difference may develop, suggesting appearance of circulatory insufficiency.[9] Experimental studies have indicated that the insufficiency is probably not myocardial dysfunction, but rather vasomotor, likely venomotor.* In these studies, infusion of noradrenalin promptly restored output to normal. The status of blood flow at 33° C. is not known, but there is likely some reduction (Fig. 2). At 30° C., significant reduction in flow (about 50 per cent) occurs, with further fall until 15° C., at which flow is minimal. Depression of circulatory reflex mechanisms develops in relation to depth of cooling, with baroceptor depres-

*E. Blair: Unpublished data.

*E. Blair: Unpublished data.

sion occurring below 28° C.[10] Vasomotor activity is impaired at 25° C. However, after short periods of cooling, these reflexes return to normal on resuscitation.

Much has been said of the increased irritability of the cold myocardium and its proneness to arrhythmia, especially the dangerous ventricular fibrillation. There is, indeed, a direct relationship between irritability and depth of cooling. However, significant alterations do not appear until the temperature has dropped below 28° to 30° C.[21] At this point, atrial arrhythmias appear and, in the diseased heart, first degree conduction block. Ventricular fibrillation or arrest can occur at any level, but the former is usually observed around 26° to 25° C. and the latter below 20° C. However, fibrillation becomes a problem only when the heart is unduly manipulated, and electric shock resuscitation is entirely adequate.[47] Of striking importance is the enhancement of myocardial efficiency, which reaches a peak at 28° to 30° C. and returns to normal levels at 25° C.[4] Myocardial contractility, as measured by strain gauge arches, is distinctly improved.[19] The coronary blood flow in relation to total body blood flow improves at all levels of cooling.[35] Myocardial oxygenation is entirely adequate up to measured levels of 20° C.[30] There is a progressive increase in total peripheral resistance with lower temperatures. Part of this is due to (1) cold pressor effect, (2) reduced blood flow, and (3) increased blood viscosity.

Blood, plasma, and constituents. Impaired coaguability, increased hematocrit, and other defects attributed to hypothermia do not appear significantly until the level of 25° C. is reached.* Similarly, blood and plasma volumes, measurements of which are not without question, do not seem to alter except at the same depth.[25] These studies are usually short-term acute experiments. The status in prolonged hypothermia, even at moderate levels, is not known. The effective circulating blood volume must be reduced at 30° C. and lower, since perfusion requirement is but 50 per cent of normal. There is much controversy over the status of serum electrolytes. Generally, it appears that significant alterations (caused by cold) occur only at 25° C. or below.* At this level, as hypothermia extends beyond 2 hours, there is a fall in serum calcium and potassium, with no alterations in sodium, magnesium, and chloride. Platner and Hosko[31] demonstrated an elevation in magnesium. A similar change was noted by Axelrod and Bass,[2] who found, however, no change in serum potassium. Plasma proteins and glucose are unchanged, as a rule, above 25° C., although hyperglycemia appears in prolonged cooling.* Serum glutamic oxalacetic transaminase levels alter only after prolonged hypothermia below 28° C.* Biochemical management in the ill patient cooled to 33° to 30° C. then would be identical to that of the uncooled patient. Deficits are corrected in the customary manner.

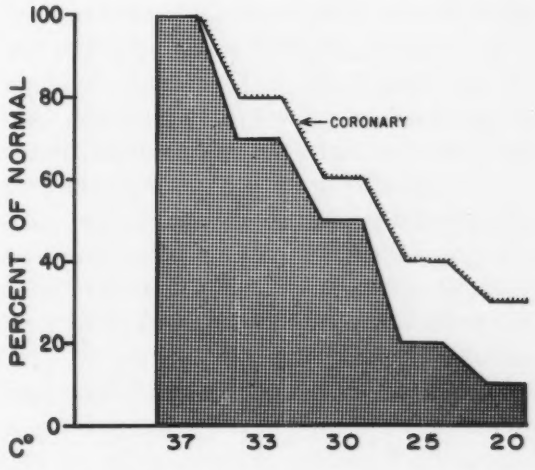

Fig. 2. Blood flow with hypothermia. Coronary flow decreases less than over-all flow during hypothermia.

Central nervous system. Utilization of hypothermia in this area is second only to

*E. Blair: Unpublished data.

*E. Blair: Unpublished data.

that in cardiac surgical procedures. As with the heart, the A-V_2 oxygen difference remains entirely within normal limits regardless of the depth of hypothermia down to 15° C., the lowest level studied.[33] Blood flow is reduced by 20 per cent at 33° C., as is the oxygen requirement, with continued linear reduction until 25° C., at which reduction is by 70 per cent. Oxygen availability is adequate through 15° C. and likely at lower levels. The electroencephalogram is active through 28° C., begins depression around 25° C., and is absent below 20° C. Brain volume is reduced and cerebrospinal fluid pressure falls.[34] In man, changes are less predictable, likely because of the type of anesthesia employed, but man nonetheless demonstrated essentially similar trends as did animals.[43] In the study of spinal cord reflex pathways by direct stimulation, cooling produces augmentation, hyperresponsiveness, and hyperreflexia to the range of 30° to 28° C., after which marked depression occurs.[23]

Miscellaneous. Renal blood flow and glomerular filtration rate progressively decline with lowering of temperature.[29] The metabolic requirement of the kidney for oxygen shows a proportionally greater reduction than does any other organ, being but 50 per cent at 33° C. There is increased renal vascular resistance along with reduction in plasma flow. The urine volume increases. The reason for this is not precisely known; however, there is an increased sodium chloride excretion, especially below 30° C., which may be in part responsible. The increased excretion is attributed to depressed tubular activity.

Table III. *Hypothermia classification*

Temperature (C.)	*Degree of functional alteration*
33°	Augmented
30°	Moderate
25°	Moderately deep
20°	Deep
Below 15°	Severe

Bile formation is reduced along with hepatic blood flow, oxygen requirement, and liver detoxifying capacity.[11] Liver volume is increased, suggesting either sequestration of blood into the liver during hypothermia or fluid shift into the cells, reversible on rewarming. Conjugation of morphine and inactivation of thiopental are reduced in proportion with reduced oxygen consumption. Glucose metabolism is reduced significantly only below 30° C., but not abolished. With prolonged hypothermia, the glycogen store becomes reduced.

Endocrine activity is reduced.[22] While most observations were made at 25° C., it is likely there is a decreased hormonal production and activity, more or less commensurate with the reduced oxygen utilization at more moderate levels of hypothermia. Circulating corticoids are down by one-half at 30° C. and by three-fourths at 25° C. ACTH is one-fifth of normal at 25° C. in the traumatized animal. Infusion of ACTH failed to increase corticosteroid production during deep hypothermia. Catecholamine actvity was relatively unchanged in the human above 30° C. In animals in the range of 25 to 20° C., the levels became immeasurable. Regional cooling of the adrenal gland yielded the same results, indicating the reduction in hormone metabolism is a direct function of cold on the gland and not secondary to total body cooling.

Little is known about immunologic response effects of bacteremia and antibody activity during hypothermia. Fedor, Fisher, and Fisher[18] have reported normal bacteria clearance and phagocytic activity by the blood stream as low as 25° C.[18]

Summary. Metabolic reduction during cooling is essentially exponential. Together with alterations in homeostatic mechanisms, in hemodynamics, and in respiration, it is possible to develop a classification of hypothermia. One such classification, with an eye toward therapeutic rationale, appears in Table III. It must be understood that the temperature levels selected repre-

sent the approximate range at which the degree of functional alteration appears. The augmented phase around 33° C. is characterized primarily by a rise in arterial blood pressure together with an increase in myocardial efficiency. Reflexes are stimulated. Heart rate falls. Ventilation is improved. There is a reduction in metabolism and a general relief of stress from undue work of breathing. At approximately 30° C., the significant depressive alterations are observed, especially in ventilation. At this level, respiratory control often becomes necessary. The range of 25° C. represents the point of significant hypotension and further decrease of ventilation. Also, first evidences of fluid and blood volume shifts, together with possible electrolyte alterations, appear. Endocrine activity is depressed. This level also is in the range of potentially fatal arrhythmias. Below 20° C., central control of homeostasis reveals marked depression, and below 15° C., signs of suspended animation such as cold heart arrest occur. If the deeper levels below 30° C. are maintained for brief periods, perhaps 2 to 3 hours, resuscitation leads to return to normal activity. At more moderate levels such as 30° to 33° C., duration of hypothermia can be prolonged even in ill subjects as long as several weeks with relative safety.

Clinical application

Cardiovascular surgery. Cooling permits a greater tolerance of hypoxia. The circulation may be arrested for periods of time enabling entrance into the heart. Experience has dictated that this procedure of circulatory occlusion is best confined to simple lesions, such as secundum atrial defects, pulmonary valvular stenosis, and congenital aortic stenosis. The range of hypothermia used is 28° to 31° C., with periods of occlusion of 10 to 12 minutes safely. This moderate range is also available for vascular surgical procedures, permitting prolonged clamping of the aorta at various levels. The hazard of renal ischemia is reduced, and tolerance of the spinal cord to hypoxia is increased. In conjunction with total cardiopulmonary bypass, two methods are currently employed: (1) Hypothermia level of 30° C., which reduces blood flow and oxygen requirements by about one-half. Thus the problem of hemolysis and accumulation of metabolites is reduced. There is also a safety measure for the brain and the heart in the event of mechanical failure of the pump. (2) Cold cardiac arrest below 15° C., which provides the advantage of requiring little or no perfusion for prolonged periods of time. A truly quiet, resting heart is obtained. Metabolic demands are minimal and tolerance to hypoxia is greatly enhanced. This cold arrest entails total body cooling. A modification provides for selective arrest of the heart with perfusion of the body artificially. The former method likely is safer.

Neurosurgery. Again prolonged arrest of the circulation because of increased tolerance to hypoxia by reducing cerebral oxygen demands is the prime rationale. Total body cooling or selective cerebral cooling may be used. The level of 28° to 31° C. is often used, which provides adequate time for cerebrovascular surgical operation. As an aid in treatment of trauma, the level of 33° to 30° C. has been found useful. Intolerance to cerebral hypoxia or trauma is manifested by progressive deteriorating signs leading to coma and decerebration.[32] This is felt to be due to cerebral edema primarily, as often seen following cardiac arrest. Hypothermia will not alter irreversible damage, but will halt or slow the progress of the edema. One of the most striking aspects of hypothermia is the awakening of the comatose patient and the augmentation of reflexes at 30° to 33° C.

General surgery. Significant reduction in bowel activity, motility, and secretion occurs from 34° to 30° C., with reduction in blood flow below 30° C. These form the bases for the treatment of gastrointestinal bleeding, using regional gastric cooling. Whether for medical treatment or for surgical operation in hepatocellular disease,

reduction in metabolic activity and in perfusion together with cold narcosis, reducing anesthesia and analgesia requirements, provide the physiologic criteria. The poor risk patient undergoing surgical operation falls into the preceding category, with the added benefit of mitigating stress response in the face of likely depleted adrenal stores and increased myocardial efficiency, urinary excretion, and central nervous system protection. In septicemia, with its severe cerebral, hepatic, renal, and vascular complications, all of the benefits derived from the augmented level of 33° C. accrue. The vascular pressor effect sustains or restores a fallen blood pressure. Reduction in metabolism with subsequent decreased perfusion requirement aids the circulation, while myocardial efficiency is increased. Urinary excretion is enhanced and the progressive cerebral edema is relieved. In trauma, there is little benefit in the face of massive hemorrhage. The vascular system already is maximally constricted. There is no substitute for blood replacement. The over-all effects are of more importance in reducing metabolic and perfusion needs of the vital organs and ameliorating to some extent risks from cerebral and hepatorenal hypoxia and a severe stress syndrome. In the therapy of burns, the shock state is aided by the pressor effect and pain is allayed by cold narcosis. Of great importance is retaining the level of cold above 30° C., for below this level, electrolyte and fluid alterations are likely to occur. The danger of further hemoconcentration is at 25° C. and not at the recommended levels.

General medicine. The principal application has been in infectious diseases, combating hyperpyrexia and toxemia. The homeostatic mechanisms are not seriously impaired by cold until the level of 25° C. is reached. Thus, the benefits of moderate hypothermia may be realized without added risk to the sick patient. In hyperpyrexia, metabolism is increased fourfold. This places a burden on a toxic cardiopulmonary system. There is severe stress, which in itself may be detrimental to the ill patient. Elimination of this high metabolic cost represents a tremendous savings to the patient, particularly if he is debilitated. The huge need for oxygen is dropped below even the normal requirement, with resultant reduction in perfusion ratio. The toxic heart is slowed, and since oxygen demand is lowered, the work of breathing is reduced. Should a shock state secondary to septicemia supervene, the cold pressor effect would assist in maintaining arterial blood presure. Hepatorenal damage may be allayed considerably. Central nervous system involvement would be mitigated as discussed previously. Bleeding problems have been mentioned earlier.

Acute pulmonary insufficiency, such as pulmonary emphysema or any acute bronchospastic state, with uncontrollable respiratory acidosis would be benefited. Need for oxygen is reduced. Carbon dioxide production is lowered, subsequent to reduced metabolism. There is greater tolerance to hypercarbia. The lungs would not have to work so hard to provide ventilatory needs at 33° C. as compared to 37° C. With acute pneumonic processes, especially in infants, all of the benefits enumerated would be derived at the augmented level of hypothermia.

Attempts at cooling patients with acute myocardial infarction would appear to have potential, but the danger of arrhythmias may be heightened. The pressor effect would aid the shock state, and the reduced oxygen requirement, the circulation in general. Coronary flow ratio is higher at lower temperatures. Marginal zones in infarction are seats of increased irritability, and a question does arise as to precipitating ventricular fibrillation with cooling under these circumstances.

Thyrotoxicosis is lessened by virtue of the reduced metabolism. Thyroxine formation is slowed, as is its action. The toxic effects of eclampsia would be allayed, for the reasons cited a number of times already.

Discussion

It is quite clear that hypothermia stands as a significant weapon in combating acute fulminating disease and in vascular surgical procedures. The crux of the entire matter is not that hypothermia serves as a specific tool. It is not curative therapy. Rather, by causing metabolic alterations and by keeping the patient out of a stressful environment, it provides the physician with a little more time to marshal his forces and apply the accepted managemental regimens with greater energy. Hypothermia, then, is an adjunct; and, as with all adjuncts, the proper management of a specific clinical situation must follow the dictum of sound medical or surgical care. The risk as a rule is insignificant, if at all present, in the augmented level of about 32° to 33° C. Below 30° C., serious depression of physiologic function occurs. The earliest serious interference is with ventilation. This level is to be avoided except for special circumstances, viz., a comatose patient already on an automatic respirator. Deeper levels of cooling are reserved for elective surgical operation where (1) the temperature is more rigidly controlled and (2) the low level is maintained for rather brief periods.

Decision to institute hypothermia in a particular situation is governed by physiologic rationale with respect to level and duration of cooling. When to cool rests with both an understanding of the physiologic impairment of the sick patient and a sizable dose of intelligent clinical judgment. The potential of hypothermia is a two-edged sword. The risk is a function of understanding the effects of cold over a broad range of temperature and of time.

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The sensitivity and validity of drug evaluations in man

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Because of divergent results with the same drugs despite what seemed to be well-controlled clinical evaluations, Houde and I examined the factors which influenced clinical evaluations. It was our conclusion, published in a Report to the Council on Drugs of the American Medical Association,[10] that, despite the proper application of double blindness, placebo controls, randomization, and statistical analysis, there nevertheless remained forces other than drug actions which could not be eliminated by these devices and which also played an important role in determining the results of an investigation of drug actions in man. In short, we found that the use of controls was not the whole story in experimental design and that powers of discrimination ultimately determine the applicability of methodology to the problem before it; that is to say, the sensitivity of the method determines whether the method itself is capable of sensing and distinguishing the differences it purports to examine and, in fact, of providing meaningful answers. We proposed, therefore, that every clinical investigation incorporate a scale of sensitivity, an indicator, so to speak, of its ability to discriminate, an evidence of its visual acuity. Failing this we suggested that regardless of the use of the above-mentioned control devices and of statistical validation, a negative answer might be without meaning and a positive one might have no quantitative significance.

Since our conclusions and suggestions are not really new to experimental methodology, nor are they especially profound, it came as a shock to find our report quoted in papers which seemed to have missed the point completely. It is apparent that we failed to communicate our conclusions. There is a justification, therefore, in an attempt at more effective communication, in restating our thesis more directly, from the point of view of how the sensitivity of methods of evaluating drugs in man is influenced by forces which operate during the course of an experiment.

There seems to be a need for such a statement for, while it is the sensitivity of the method, the responsiveness of the measuring instrument, which determines the meaning of the results of an experiment, the details of what determines it is, nevertheless, not ordinarily a part of our medical curriculum. This is not to say that there are no high standards or good methods, for we have both,[8] but the necessity for using them is not always recognized or even stressed. This is a sad deficiency for there are many new drugs and many poorly designed reports on them. Often conclusions of investigations are accepted as published without question and no attention is paid to whether the investi-

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gations are competent to detect what they conclude. Thus a large number of papers on new drugs contain claims which are nevertheless swallowed whole without digestion and their recommendations promptly followed without reservation. Unless the physician himself has some knowledge of what determines the sensitivity, the reliability, the applicability of methods of drug evaluation, in the case of reports on new drugs, only the presumably well-intended but, alas, certainly fallible, journal editor stands between him and reliance on shaky or spurious claims.

The issues for resolution in the clinical evaluation of drugs are basically the same as for well-designed experiments in all other experimental disciplines and can be stated simply enough: identification and control of all the factors which may interfere with or assist in making observations and collecting and evaluating data. This is what I want to consider in outlining how sensitivity is affected in clinical evaluations.

For a simple explanation I propose to use as a model scales which weigh the evidence for and against drug action. In a proper clinical evaluation the effects of drugs per se are matched against all other influences which tend either to prevent the action of the drug from swinging the balance in the proper direction or to tip it in the opposite direction. In either case the scales are used to provide answers which are not due to some active force other than drug and which otherwise might be misinterpreted as being evidence of drug action. What determines the sensitivity of these scales, hence whether the results are meaningful or meaningless depends, therefore, as much on what is put on one side of the balance as on the other, as well as on what difference between the weights on both sides will swing it.[10,12]

Factors which influence patient response

The factors which influence the sensitivity of methods of clinical evaluations may be enumerated as follows: (1) the pharmacodynamic action, (2) the dosage, (3) the subject, (4) the controls, (5) placebo actions, (6) the forces extraneous to the experiment, (7) the method of collection of data.

Pharmacodynamic action. When objective measurement of effects is possible, and when pharmacodynamic actions are potent, reproducible, and are not significantly influenced by psychic forces, precise evaluation is relatively simple. Drug actions which must be evaluated in terms of subjective responses, and especially those which are not in themselves impressive, are far more difficult to measure or even estimate. Drug actions on function, which, like blood pressure, have a tendency to wide spontaneous variation or are altered by immediate circumstances, such as tension, position, strain, etc., provide serious problems in assessment.[1]

Dosage. The proper measurement of drug action requires the use of the proper dosage. It is obvious enough that when dosage is too low, regardless of the pharmacodynamic actions or potency of the drug, no method of clinical evaluation can reveal a difference between drug and placebo, and when the dosage is too high therapeutic effect will be obscured by a toxic effect. Neither toxic nor token dosage may be used in clinical evaluation.

The subject. In much the same way that some species of laboratory animals are superior to others for particular experiments in the laboratory, the choice of a suitable subject is often a critical matter for an investigation in man. Thus, while the best subject will tend to make the method more sensitive, unsuitable subjects may dilute the response to drugs and make the method so insensitive that it is unable to detect the particular drug action under investigation and, therefore, regardless of the activity of the drug or effectiveness of the controls, provides only a negative answer.

Subjects must be selected in a manner which insures the ability of the group as a whole to discriminate between active and inert agents; that is to say, the subjects

must be sufficiently sensitive to the drug action under investigation to be able to discern relatively small differences in effect. In studies involving subjective criteria, exceedingly phlegmatic subjects tend to desensitize the method by failure to react with normal intensity while exceedingly neurotic and over-reactive or highly suggestible patients tend to compromise the sensitivity of the method through wide swings of mood and attitude as the result both of placebo and of active medication. In general unusual and abnormal as well as hypersensitive and resistant subjects desensitize evaluations.[13]

If the evaluation is to have predictive value concerning the therapeutic uses of a drug, the group of subjects must represent a fair or random sample of those who will use the drug, whereas if the purpose of the examination is to determine its pharmacologic actions, normal subjects or patients may be employed.[10]

If an indiscriminate group of subjects is used for drug evaluation and the problems of chance differences in the subjects are dealt with merely by the process of randomization, this simply equalizes the influence of a large amount of dead weight for, in order to overcome the spurious swinging of the balance, an equal number of irrelevant responses of unsuitable subjects are put on both pans. However the choice of the subject is finally made, it should be with the idea in mind that his proper selection has a great deal to do with the ultimate sensitivity of the method.

The controls. There is no such thing as an uncontrolled drug evaluation, for the control is the basis of all comparisons, the standard for the statement that the result of the experiment is positive or negat Drug evaluations which fail to provide clear-cut controls, consciously or unconsciously, rely on recollection or someone else's experience, both treacherous. In any event, despite any disclaimer every experiment *has* a control; some are satisfactory, some are hopelessly inappropriate and provide the basis for misleading conclusions.

Matching of control and experimental human subjects is an insuperable task. The classic clinical experiment employs separate groups for control and treatment, but this provides significant data only when the groups are formed by random selection and when they are large or extraordinarily homogeneous. An acceptable alternative is to give each patient medicament and placebo so that each subject serves as his own control, the so-called cross-over design.

In any event, the more homogeneous the subjects and the more constant the circumstances for both control and experimental groups, the more sensitive the method and, conversely, the more variable the subjects and circumstances which must be equated by the process of randomization, the more insensitive the method.

Placebo actions. The term *placebo*, has taken on many implications not within the philologic meaning of the words,[6] for example, *negative* (i.e., undesirable) placebo actions.[14] As the word is currently used in clinical evaluations, it includes a complex of visceral, somatic, and psychic responses to the physician, to his presence, to his word, to his ministrations, and to his medications. Such an action is inherent in all medication regardless of whether it is useful, hazardous, impotent, inert, unpleasant, inadequate, or inappropriate, or, for that matter, new or old, as long as it is prescribed by the physician.[7,11,15]

Since there is no escape from placebo action, to be certain that this is not the only effect of a drug under examination, it is absolutely essential to have some means of indicating that the effect of the drug is in excess of "pure" placebo effect. Such a measure provides the only defense against the suggestion that results reported after the administration of a drug are due only to placebo actions. In using a placebo control it is well to recognize that in the analogy provided by the balance the placebo effect is not restricted to one pan and the drug action to the other. Since placebo action is inherent in every act of medicating by the physician there is, in fact,

placebo in both pans, and the scales merely measure the difference between them. That is to say, placebo effect is exerted on both pans at all times, and the only measurement is of that which the drug may provide in excess of its inherent placebo action and, in the event that the two do not summate but overlap, it measures merely drug action which is not masked by placebo action.

Bias. In addition to the considerable psychic force exerted by the administrator of a drug, particularly if he be a physician, the so-called placebo action of drugs, the hopes of both the patient and the therapeutist, as well as any bias either may have with respect to treatment or experiment, also exert considerable force on patient response after the administration of drugs. Therefore, all clinical evaluations must reckon with bias.

The patient may want to get better to the extent that he is inclined to see good effects after administration of new medication and color his subjective responses accordingly. On the other hand, he may find compensation in his illness and wish to preserve his complaints, hence be inclined to deprecate pharmacodynamic effects. The physician's knowledge of the nature of the medicament is exceedingly important for, regardless of how much he tries not to, he may nonetheless relay information to the patient and influence him. In addition, the physician's understandably hopeful attitude to treatment may lead him to interpret, hence modify, data along preconceived lines as he collects them and, as a result, there may be substantial apparent effects from accumulated bias.

The double blind technique is a philosophically sound as well as a practical control device to deal with the tendency of conscious and unconscious bias to obscure and distort the observed effects of drugs. It deals with the influence of the physician's professional purpose to help his patient as well as his preconceived ideas and prejudices about the medication and his unconscious communication to the patient on his observations by blinding him, that is, keeping him ignorant of whether he is giving or has given his patient placebo or active drug. Likewise the effects of the patient's hopes and his anxieties on his psychic and somatic responses to drugs as well as on his estimates of his subjective responses are dealt with by blinding him, that is, keeping him ignorant of whether he is receiving or has received drug or inert medicament of identical appearance; hence the double blindness.[5]

What is important to remember is that the myopia and astigmatism of the physician and the subject due to bias are corrected only in the sense that blinding will compensate for them, and nothing is done to increase the visual acuity of either participant in the experiment. As already stated, double blindness does not eliminate bias as an element in the method; it merely deals with it by equalizing its effects so that, as weighed in the scales, unequally distributed or accumulated bias alone will not account for an apparently decisive result. While it prevents an erroneous positive result it does not increase the sensitivity of the method or prevent erroneous negative results.[10]

External forces. There are a large number of extraneous influences which affect the state of the subject's physical, functional, and psychic state—a change in the course of his illness, a happy experience, a lost job, a family quarrel, a seasonal allergic state, a change in the weather, a turn in world affairs—which may also influence his response to drugs.[2] These may be both objectively and subjectively recorded responses.

The tendency of external forces to influence response can be dealt with to a limited extent only by reduction of the every-day disturbances of living through hospitalization or careful nursing or other forms of protection for the patient. What remains must be dealt with by prescribing medication and placebo by a scheme of randomization so that the disturbing forces affect the apparent response to placebo and drug

alike, and being equally spread they favor neither.

Collection of data. When objective measurement is possible and differences can be expected to be substantial, there is relatively little difficulty in the collection of data. When the patient must communicate his subjective experience, however, it is quite another matter. There are few experimental procedures in which so vital a part of an experiment as the collection, storage, and interpretation of observations is put in the hands of an interested, biased, and untrained assistant, yet this is precisely what is being done where the patient-subject is asked to report and summarize his experiences after a period of medication. How can he help having his recall affected more by recent events than by those farther back in his memory.

The daily report-card system was designed to deal with this defect in the interval report system by decreasing the interval between the recording of responses to one day. While this method of data harvesting obviously supplies more data than a longer interval report system, it has not been subjected to an analysis which proves that the data themselves or the answers derived through their use are more substantial. Other improvements have been suggested: having the subject mail a postcard each night or telephone a report each night,[4] but even these compromises leave the data subject to the caprice of the patient for too long.

Houde and I showed in our Report to the Council on Drugs that any device which leaves the discrimination of the reaction to drugs at the mercy of patient recall increases the possibilities of outside influences tampering with data and thereby decreases the sensitivity of the method. Every effort should therefore be made to minimize the period between the experience with the drug and the recording and collecting of data; the data should be taken out of the patient's hands as soon as possible and, thereby, kept as nearly as possible in the original form. As time and attending circumstance are permitted to affect data, once more the process of randomization burdens both sides of the balance with yet more dead weight, equally distributed, to be sure, but nevertheless weight immaterial to the problem at hand, which is the weighing of pharmacologic actions only.

The implications of the balancing

Which way the scales which we have used as the model for methods of drug evaluation swing, that is, whether drug action vis-à-vis chance is favored, depends, of course, on the relative total weight in one or the other pan. Whether the swing of the balance or its failure to swing is meaningful or misleading depends on whether this result is the outcome of a specific action of the drug or of one or more of a myriad of forces which influence man's behavior and his mental, physical, and visceral activity. When this model is used, one way to prevent swings of the balance by forces other than the intrinsic pharmacodynamic action of the drug itself is to spread them equally on both sides of the balance, thereby causing no disturbance in balance by their influence. This is what is accomplished with the control devices of double blindness and randomization; they merely prevent chance or biased swings of the balance in either direction.

What is rarely taken into account in clinical evaluations is how much weight is necessary to make the balance swing at all, that is, the basic sensitivity of the method. Whatever the original sensitivity of the balance, consider what is done with it by accepted and essential control devices at hand. Consider that the scales are not empty at the outset of the drug evaluation, merely in balance (Fig. 1). Equally distributed on both pans are placebo action of drugs, bias, the influence of diverse extraneous factors such as weather, political events, family stresses, and a number of other vagaries of human experience that tend to mold or alter man's functional state and his response to drugs. It is to be re-

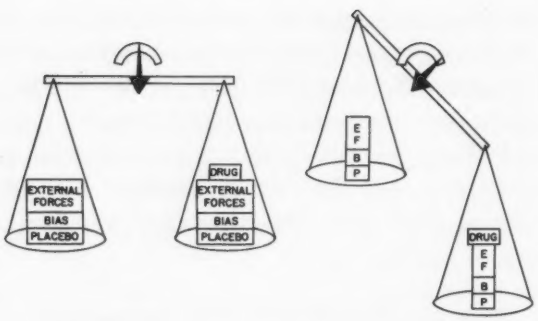

Fig. 1. Diagrammatic representation of experimental designs for clinical evaluation. *Left:* Excessive dead-weighting to prevent chance occurrence from swinging the balance reduces sensitivity to the extent that drug effects do not swing balance and, hence, cannot be measured. *Right:* Reduction of dead-weighting through appropriate design makes it possible to measure pharmacodynamic effects of same intensity with same balance. (From Modell and Houde, J.A.M.A. **167**:2190-2198, 1958.)

peated; these are not removed, they are accepted and spread equally over both pans of the balance by the process of randomization and by the control of double blindness. The balance is thereby "dead-weighted" with a large amount of material which is foreign to the particular problem at hand. No matter how sensitive originally, such a procedure makes a balance less sensitive just as an analytical balance sensitive to a fraction of a milligram under usual conditions is not swung out of balance by a few milligrams after "dead-weighting" with several kilograms on each pan.

Ultimately, therefore, the sensitivity of a method of clinical evaluation is a function of the relative weight of the pharmacodynamic force under investigation and the weight of the interfering forces which are treated by equalization; the greater the former with respect to the latter the more sensitive the method and, vice versa, when the latter becomes relatively heavier, the method becomes proportionately less sensitive. To the extent that "dead-weighting" grossly desensitizes, the process can lead to erroneous interpretations in that it provides a negative result whenever a method is used to detect differences in effect which it can no longer sense.

Of the disturbing factors already discussed, some are subject to choice; these may be eliminated. Thus it is usually possible to choose the proper dosage range. Sometimes it is possible to obtain the most sensitive subjects and the best possible controls for the study. Some factors which cannot be eliminated may be modified; e.g., the removal of the patient from the home to the constant environment in the hospital may reduce the external variables while the collection of data on the spot reduces the treachery of patient recall. Finally, there remain some which cannot be reduced, removed, or modified: bias and placebo actions. For these and extraneous forces which cannot be eliminated there is only the double blind control and randomization to spread the prejudicial effects equally.

As in methods for chemical analysis, every design for drug evaluation requires a demonstration that its sensitivity is appropriate for the distinction it chooses to make. A scale of sensitivity should indicate, first, the ability of the method to detect the drug action per se and, second, the increments in effects which it can distinguish. The first gives meaning to the negative answer and the second gives quantitative significance to the positive answer. A negative answer is valid only if it is demonstrated at the same time that the method can also discern the effects of a standard similar drug. The ability of a method to discriminate increments in effects can be indicated by its capacity for dosage response when a series of graded doses of the standard or experimental drug is used.

A great danger in interpreting clinical evaluations lies in failure to recognize the meaninglessness of the negative answer when the method is not sufficiently sensitive for the purpose. A failure to demonstrate statistically significant differences between drugs or treatments is difficult to assess. However reasonable it may seem

from the data, an assertion that drug and placebo effects are identical is not easily proved. Statistical tests of significance merely indicate a likelihood that whatever *differences* are noted in the data are due to chance. Thus, when the differences are statistically significant it is unlikely to be a chance occurrence, and with a measurable degree of confidence, rightly or wrongly (for the statistics themselves do not validate the basic data), they can be ascribed to differences in the effects of the agents under investigation. On the other hand, differences which are not statistically significant could result simply from an inadequate trial or, what statistical analysis may not indicate, from an insensitive method of evaluation.

It is well to remember that statistical analysis proves nothing about the original validity of the data—it is merely a device for establishing the betting odds on the reproducibility of the results obtained by the same method, the probability of obtaining similar results with future experience under the same conditions. Statistical prognostication is always based on the assumption that the data used were worthy of collection; statistical analysis of poor data cannot provide first class answers.

It should be made clear also that, although statistical procedure presently seems to have assumed an especially prominent position in reports on drugs,[3,9] fundamentally its use is not at all new to medicine. As with the use of controls, no matter how an experiment is planned, how the terminology seems to intrude, or how the results are expressed, the problems of variability and the possibility of chance occurrence are inseparable from the significance of findings in the clinical evaluation of drugs. It is a biologic fact that all human and animal reactions and failures to react exhibit individual variability and, as a consequence, any statement about the pharmacologic or therapeutic action of a drug has implicit in it also the statement that it is not a chance occurrence falling within the range of biologic variation. Such a statement about the effect of a drug is, therefore, based on either a calculation or a guess of probable or statistical significance; the only questions which remain are its quality and its applicability. This is not to say that a statement of statistical significance insures correctness of an interpretation; if the data are inappropriate or improperly collected, despite their statistical significance, an interpretation may nevertheless be erroneous.

Conclusions

Clinical evaluations are so beset by external forces which may affect them that every possible control measure must be applied if valid and durable results are to be obtained. Every effort must be made to insure the suitability of the method of exploration. The selection of the proper dosage range is vital and the selection of the proper subject is equally critical in the design for clinical evaluation. As far as possible, all external disturbances should be eliminated. Data must be collected promptly. Treatments must be randomized. In addition to the use of the placebo, the double blind control should also be used whenever and wherever it is feasible. It will prevent false positive interpretations but it cannot prevent a false negative result. There is no conceivable disadvantage in the application of the double blind control, only protection against spurious data. However, it must be used to deal only with bias and psychic forces which cannot be otherwise eliminated, for its use will not validate otherwise poorly designed experiments.

The use of the appropriate controls merely serves to prevent extraneous forces from swinging the balance erroneously; not only does this fail to increase the sensitivity of the method, but if used merely to balance out extraneous influences by the process of "dead-weighting," it may even make it less sensitive. To increase method sensitivity it is necessary to *remove* biasing factors.

Each clinical evaluation must be sensitive enough to detect what it proposes to

discover, and each experimental design must have built into it an indicator that it is capable of such detection. A negative conclusion is without merit unless there is incorporated in the clinical evaluation a demonstration that the method is competent to indicate a positive effect when it is present, i.e., an internal control. It is suggested that in clinical evaluations another demonstrably effective drug always be used in addition to the placebo control to indicate this essential competence of the method. The complete clinical evaluation must also include a built-in sensitivity scale and, through the use of graded doses, a demonstration of the increments in pharmacodynamic effect which the method can distinguish. When differences between standard and unknown or placebo are indicated, the sensitivity of the method to distinguish differences is thereby at hand to indicate the quantitative significance of the differences.

The definition of the effects of many drugs and the proof of the superiority of one drug over another require investigational designs which are based not only on the principles laid down here but which are also designed with due regard to the particular drug, the particular subject, and the particular circumstance as well as the specific action under investigation. There is yet no standard method—there are basic requisites, essential controls, and some well-established procedures, but each different pharmacodynamic action may need a different subject, a different control, a different circumstance, or a different design for its proper evaluation.

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Symposium on the experimental pharmacology and clinical use of antimetabolites

Part III. Metabolic antagonists and selective virus inhibition

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The specific biochemical effects of metabolic antagonists can be studied in a great variety of biologic systems both in vivo and in vitro, and through their use much has been learned about the biochemical processes in the various systems.[50,81] The general principles governing the biochemical mechanism of action of metabolic antagonists have been adequately set forth.[50,81]

However, when considering antimetabolites as possible therapeutic agents, it is important to distinguish two different biologic aspects of metabolic antagonism:

1. In some conditions, it is desirable to counteract the effect of metabolites which are normally present in the organism and which play important roles in physiologic processes. For example, succinylcholine, an effective antagonist of acetylcholine, is useful as a muscle relaxant drug.[4] Several contributions to the present symposium deal with metabolic antagonists in this category.

2. In other situations, it is desirable to counteract the growth and multiplication of viruses or bacteria which have invaded the host organism. To obtain effective inhibition of these agents without damage to the host organism, it is necessary to inhibit metabolic processes of unique importance to the invaders—processes which the host organism does not require. The action of the antagonist should be aimed at a specific biochemical feature or metabolic requirement of the invader, i.e., the antagonist must possess selective toxicity for the invader.[2,81]

The main purpose of this article is to summarize the available information on the specific biochemical features of viruses and to report recent progress which has been made in studies on selective inhibition of virus multiplication. Only secondary attention will be given to those requirements and properties which viruses share with their hosts, and little will be said about metabolic antagonists which inhibit virus multiplication through inhibition of one or more of the many host cell processes on which viruses depend for their reproduction.

Virus life cycle

Nature of viruses. Viruses are particles of small size and simple structure.[7,57] They are naturally transmissible from cell to cell and are capable of self-reproduction within

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susceptible host cells. Viruses are also capable of variation of heritable properties and of selective survival within the appropriate ecologic system. Viruses vary in size, shape, chemical composition, stability in the extracellular state, immunologic properties, reactivity with cells and cell components, and ability to cause disease. However, the fundamental features of their make-up and the basic mechanism by which viruses multiply are similar in all virus-host systems. All viruses contain nucleic acid and protein; some also contain certain carbohydrate or lipid material. The nucleic acid in some viruses is of the desoxyribonucleic acid type (DNA), while in others it is ribonucleic acid (RNA). In all viruses the nucleic acid appears to be located in the interior of virus particles, surrounded by protein. Production of new virus in appropriate host cells is initiated and directed by nucleic acid released from virus particles.

Extracellular virus. In the extracellular environment, virus particles are in many respects inert.[66] Extracellular virus particles remain infective for variable periods of time depending on environmental conditions.

In studies on virus inhibition, it is important to know whether the substance under study has a direct inactivating effect on the infectivity of particles. Such inactivation can be readily detected and measured by incubating a fairly concentrated virus suspension with the compound, diluting the mixture, and determining the number of remaining infective units of virus. The infectivity of the treated virus suspension is then compared with that of the control virus preparation which was incubated in the absence of the compound.

None of the compounds or substances to be discussed below have a direct inactivating effect on the infective property of viruses.

Steps in virus multiplication. The interaction which occurs when an infective virus particle encounters a fully susceptible host cell is characterized by six steps: (1) adsorption of virus to cells, (2) penetration into cells, (3) disassembly of infecting virus and separation of virus nucleic acid (eclipse), (4) synthesis of virus precursor materials, (5) assembly of new virus particles, and (6) release of virus from cells.

Steps 1 to 4 represent the "latent" phase in the sequence of virus reproduction, since during this phase, the replicating virus cannot be demonstrated in the form of virus particles. Step 5 represents the rapid increase phase. In many instances increase in new virus is exponential for a period of time.

Disassembly of infecting virus may occur prior to penetration; indeed, not all parts of the virus particle may enter the host cell. Release of virus from cells may be fast and complete or slow and incomplete. Virus may be released into the extracellular space or it may be transmitted directly to a neighboring cell.

The fundamental experiment in studies on virus inhibition is concerned with the determination of yield of virus from infected cells incubated in the presence or absence of the substance under study. Yield from treated cultures is compared with that from untreated controls. Reduced yield in treated cultures is taken as evidence that the substance employed inhibited virus multiplication, provided the substance had no direct inactivating effect on the infectivity of virus particles.

The finding of reduced yield in the presence of an inhibitory substance does not by itself provide information as to which step in the reproductive sequence of the virus was affected by the substance. Studies on the kinetics of inhibition are necessary to secure such information.

Virus-induced cell damage

In Nature, virus multiplication culminating in the production of new infective virus particles is a prerequisite for the development of virus disease.[27,28] However, multiplication of a virus in the host organism does not always or necessarily lead to virus disease. At the cell level, virus multiplication may or may not lead to cell damage.

Cytopathologic changes can be experimentally produced with certain products of virus reproduction which are separable from the virus particle.[15,52]

These findings point to the likelihood that in many instances cell damage, and ultimately virus disease, is due to the products rather than the process of virus multiplication. This view is supported by the observation that virus-infected cells, destined to degenerate and die, may show few abnormalities and may perform normal functions early in the infection when synthesis of new virus materials is taking place. It is also clear that injurious materials produced in virus-infected cells may sometimes represent entities other than whole virus particles. However, in some instances, the process of virus multiplication may itself lead to cell damage through drain of the cell's metabolic resources.

Primary viral effects may lead to multiple secondary reactions in host cells. Indeed, it is probable that in a number of virus infections, the infecting virus triggers a pathologic reaction pattern which may be characteristic of the particular cell type or may be common to different cell types. The nature of these effects may change in relation to sequential stages in the reproductive cycle of a virus.

In recent years, information has been rapidly accumulating concerning the metabolic and morphologic changes in virus-infected cells.[57,66] However, it is premature to propose specific biochemical hypotheses for the pathogenesis of virus diseases or to speculate about possible chemical means to counteract virus-induced changes after they have occurred.

A substance capable of inhibiting selectively the multiplication of a virus may prevent viral damage to cells. However, an inhibitor of virus multiplication may be expected to do so only if it is administered prior to the occurrence of the step or reaction which starts the irreversible chain of of infected cells. If only a small proportion events leading to degeneration and death of cells is infected at the time of administration of an effective inhibitor of virus multiplication, spread of virus may be prevented, but some or many of the cells already infected at the time of addition of compound may degenerate and die.

Addition of a nonselective virus inhibitor to an infected cell population is not likely to improve the chances of survival of infected cells. Although virus multiplication may be inhibited, the inhibitory effects of the compound on the metabolic activities of the cells themselves may greatly outweigh the advantages of suppressed virus reproduction. In fact, degenerative changes may be accelerated in infected cells incubated in the presence of a metabolic inhibitor lacking biologic selectivity.

Biochemistry of virus multiplication

Virus multiplication is intimately dependent on host cell metabolism. Virus nucleic acid provides a genetic influence which causes new virus particles to be synthesized in susceptible host cells. The host cells provide the necessary building blocks, enzymes and energy; in addition, host cell RNA appears to play an essential role in virus synthesis.[68,75] The intimacy of the virus-host relationship is emphasized by the fact that synthesis of new virus materials takes place in the host cell protoplasm in the absence of limiting viral membranes.

Chemical basis of virus specificity. The main product of the process of virus multiplication, i.e., the virus particle, possesses many distinguishing biologic and chemical features which are characteristic for each particular virus. The main components of virus particles, namely the nucleic acids and proteins, also possess virus-specific features. For example, virus nucleic acid derived from infective virus particles, when allowed to react with susceptible host cells, is able to initiate the production of whole new virus particles of the kind from which the nucleic acid was originally derived.[11] Virus proteins are immunologically specific and distinct from host cell proteins.

Two questions may be asked: (1) What

is the chemical basis of specificity of virus nucleic acids and proteins? (2) What steps in the synthesis of virus nucleic acids and proteins are responsible for the specific features and properties of these virus components?

Viruses appear to be made of the same building blocks as host cells, with the following single exception: in the DNA from bacteriophages T2, T4, and T6, hydroxymethylcytosine replaces cytosine or its 5-methyl derivative.[82] In this exceptional instance, virus infection leads to the production in host bacterial cells of several new enzymes concerned with the synthesis and reactions of the special base.[60] This base has not been found in other bacteriophages or in animal viruses, neither have any other special bases been found as yet in any of the viruses examined.

The hypothesis is widely held that the specificity of nucleic acids and proteins resides mainly in the sequence of nucleotides and amino acids, respectively, in these large molecules. The sequence of nucleotides has not yet been determined in any nucleic acid. Considerable effort is being spent on finding ways to solve this difficult problem. The sequences of amino acids in two proteins, i.e., insulin and ribonuclease, have been established and work is in progress on the structural chemical basis of the biologic activities of these proteins.

The proportions of nucleotides in virus nucleic acids vary among different viruses, but in closely related viruses component nucleotides occur in similar proportions.[49] The amino acid composition of closely related virus strains is in general similar. However, mutant strains may differ markedly from parent strains in amino acid composition.[46] A detailed understanding of the chemical basis of specificity of virus nucleic acids and proteins is lacking at the present time.

As to the steps in the synthesis of nucleic acids and proteins at which molecular specificity may be acquired, there is good evidence that this does not occur at the level of synthesis of low molecular precursors. These appear to be common to virus and host.

It is probable that specificity is imparted in the assumed interaction of virus nucleic acid from infecting virus particles with appropriate precursors provided by the host cell. According to current theory, virus nucleic acid, acting as a template, causes polymerization and patternization of precursors leading to the formation of molecules identical to the infecting nucleic acid. The precise mechanism of the template action of nucleic acids in their self-reduplication is not known.

In the final step in protein synthesis, too, template action is presumably involved. It is believed that RNA commonly serves as a template in protein synthesis.

It is probable that virus-specific mechanisms operate not only in the synthesis of large molecular virus precursor materials but also in their assembly into virus particles.

Approaches to selective inhibition of viruses. In the light of the foregoing considerations, it seems unlikely that metabolic antagonists which interfere with the production or utilization of low molecular precursors of nucleic acids or proteins or with energy metabolism might act as virus-specific inhibitors. Quantitative differences may exist in the biosynthetic demands, conditions and rates which may render virus multiplication more susceptible than the host cell to the effects of certain metabolic antagonists; however, the antagonists would probably still have significant effects on host cell metabolism at virus inhibitory concentrations.

Furthermore, approaches to selective inhibtion of the template action of virus nucleic acids are not evident at the present time. If, however, the mechanism of template action or the sequence of the repeating units were known, it might be possible to design inhibitors which would interfere with the assembly of precursors into virus materials without interfering with the synthesis of host materials of similar kind. Such information has yet to be obtained.

The chemistry of biosynthesis of nucleic acids and of proteins is in its infancy, but much progress may be expected within the next decade.

In summary, the theoretic possibilities for the development of metabolic antagonists with highly selective virus inhibitory activity do not appear good as far as the immediate future is concerned.

However, it should be emphasized that metabolic antagonists are proving useful in the study of the requirements and mechanism of virus multiplication. Furthermore, unexpected discoveries of virus inhibitory substances with significant selectivity have given promise of new approaches to the problem of unraveling the mechanism of virus specificity.

Metabolic antagonists as virus inhibitors

Because numerous recent reviews are available on the subject of virus inhibitors of various kinds, including metabolic antagonists, it seems needless to review again the large number of reports which have already been carefully discussed. In the existing review articles different aspects of virus inhibition have been emphasized by different authors. It therefore seems pertinent to indicate briefly the main features of each of the reviews. Following this, certain conclusions are presented concerning metabolic antagonists as virus inhibitors. These conclusions are based largely on the information and concepts contained in the reviews, though some newer data are also utilized.

Recent reviews. Matthews and Smith[51] emphasized the structure and multiplication of viruses in relation to virus inhibition. Among the compounds discussed, purine and pyrimidine analogues received the most detailed attention. It was pointed out that some probably interfere either with the incorporation of bases into nucleic acids or with the formation of the bases from precursors, whereas other analogues are themselves incorporated into nucleic acids and appear to exert their growth inhibitory effect through such incorporation. The mode of action of analogues which are incorporated into the end product and their possible usefulness in the control of viruses were discussed.

In the review by Hurst and Hull,[37] a very large number of compounds were discussed and separate sections were devoted to observations made in tissue culture, in the chick embryo, and in the mouse. The highly exacting requirements for a drug which would be useful against virus diseases in the field were underscored. It was also emphasized that modified infection in animals whose state of health has been impaired by administration of toxic doses of a chemical substance should not be accepted as evidence of successful chemotherapy, since an animal weak or sickly from any cause may respond atypically to a virus infection.

Horsfall[29,30] considered virus inhibition in relation to the mechanism of virus reproduction and emphasized the importance of the rates at which virus reproduction proceeds and disease develops. Compounds which inhibit intracellular reproduction of virus were considered to be of greatest interest. Horsfall emphasized the fact that multiplication of viruses is more effectively inhibited the earlier an inhibitory compound is given. In a later paper,[31] Horsfall pointed out that relatively few substances have been studied with sufficient thoroughness in regard to the kinetic aspects of multiplication to make it feasible to decide at what step in the process they are active. Those compounds which appear to affect processes that occur during the period between virus adsorption to cells and appearance of new virus, i.e., during the "latent" period, were discussed in detail.

Francis[17] discussed the multiple sites at which viral infection, proceeding from benign cellular involvement to severe disease, theoretically may be inhibited. It was emphasized that more attention should be paid to identification of the specific substrate of the virus action, for it may prove possible to prepare protective inhibitory analogues to such cellular components.

In an article by Horsfall and Tamm,[33] the possibilities of chemoprophylaxis and chemotherapy were considered in relation to the biologic background of virus infection, and an analysis of the biologic and biochemical principles of inhibition of animal virus multiplication was presented. Results of extensive studies with benzimidazole glycosides were used to illustrate numerous aspects of the problem of inhibition of virus multiplication from the viewpoint of inhibition of biosynthetic processes.

Tamm[65] presented a comparative analysis of the characteristics of viruses, Chlamydozoaceae, Rickettsiae, and bacteria in relation to availability of effective chemotherapy. Those virus inhibitory compounds which had been shown to be active in animal experiments were reviewed. Special emphasis was placed on benzimidazole derivatives because of demonstrated possibilities of improvement of virus inhibitory effectiveness of benzimidazoles through chemical synthesis of new derivatives. The mechanism of virus multiplication is the starting point of a later article by Tamm.[66] Reports of virus inhibition were selected for review if the effects of compounds on the metabolic activities of host cells had been studied. Such effects were discussed in detail. Available information concerning the relationship between structure of benzimidazole derivatives and their inhibitory activity was summarized. Some outstanding problems in selective inhibition of virus multiplication were presented. In another article,[67] Tamm discussed the applicability of the principle of antimicrobial chemotherapy to viruses. It was pointed out that this involves the problem of whether highly selective virostatic or virocidal compounds, if found, would be useful in treatment of virus diseases of man. On analysis of the biologic background of virus diseases, it appeared such compounds would probably be useful in some but not in all virus diseases. A selective review of experimental studies on virus inhibition included representative examples of virus inhibitory compounds which acted through inhibition of nucleic acid or protein synthesis or through interference with energy-yielding mechanisms. In addition, the discussion included certain compounds that act by unknown biochemical mechanisms but are of considerable interest as virus inhibitors in that they show selectivity.

Conclusions. The evidence summarized in the reviews and some newer information can be used as a basis for certain conclusions concerning metabolic antagonists as virus inhibitors. Studies with metabolic antagonists have contributed importantly to knowledge of metabolic requirements of virus multiplication. For example, with the aid of suitable metabolic antagonists, it has been shown that whereas host cell RNA plays a decisive role in the multiplication of DNA-containing viruses, host cell DNA does not appear to be of importance in the reproduction of RNA-containing viruses.[68,75] Also, it has been possible to gain insight into the time course of virus-related nucleic acid and protein synthesis in infected cells, and some information has been secured on the energy requirements of virus reproduction during the several stages in the sequence of virus reproduction.

Significant differences have been found among virus inhibitory antagonists in selectivity of action and among viruses in their susceptibility to inhibition.

However, all virus inhibitory antagonists have shown some effects on host cell morphologic condition or metabolism wherever such effects have been looked for. This observation strongly supports the view that presently available metabolic antagonists interfere with the synthesis or utilization of low molecular precursors common to host and virus or with cellular energy metabolism which supplies both host and virus. Although many virus inhibitory antagonists are chemically highly selective in that they inhibit only certain reactions when the concentration used is appropriate, these antagonists have shown little biologic selectivity with respect to viruses.

At present, there is no evidence that a metabolic antagonist which inhibits bio-

synthetic or energy-yielding processes may protect cells against the cytopathogenicity of the infecting virus. For example, in the following instances no significant diminution was observed in virus-induced cell damage although virus yield was markedly inhibited: (1) influenza- or poliovirus-infected monkey kidney cells treated with 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole(DRB), an inhibitor of RNA synthesis,*[73] (2) herpes simplex–infected HeLa cells treated with 5-fluoro-2-deoxyuridine (FDUR), an inhibitor of DNA synthesis,† and (3) poliovirus-infected cells treated with *p*-fluorophenylalanine, an analogue which is incorporated into proteins.[1]

If protection of cells rather than reduction of virus yield had been the criterion of successful virus inhibition in the numerous studies which have been published, likely few claims would have been made that virus inhibition had been achieved.

Selective virus inhibitors

Although there is no known instance of inhibition of virus-induced cell damage by a well-defined metabolic antagonist, a few chemical compounds and biologic substances have been shown to possess protective activity against viral cytopathic effects. In the discussion that follows, attention will be given to one synthetic chemical compound, 2-(α-hydroxybenzyl)-benzimidazole (HBB), to two mold products, M-8450 and helenin, and to interferons, which are proteinlike substances produced in cells exposed to infective or inactivated viruses. Each of these agents protects cells in culture against viral damage and in addition possesses some protective activity in vivo. The protective effect appears to result from inhibition of virus multiplication in the presence of these agents.

Synthetic chemical compounds

Since 1952, benzimidazole derivatives have been studied intensively as inhibitors of virus multiplication.[14,26,64-67,69-71,73-77] Extensive studies on the relationship between chemical structure and virus inhibitory activity with both existing and new compounds led to the recognition of certain patterns in this relationship and to the synthesis of new, highly active inhibitors of RNA biosynthesis. These inhibitors are ribosides of halogenated benzimidazoles and they have proved very useful in studies on the requirements and mechanism of virus multiplication.[66] Among such derivatives, the riboside of dichlorobenzimidazole (DRB)[71] has been studied most intensively, and it has been shown that DRB acts as a metabolic antagonist of adenosine or a related compound.[68,75] All viruses tested have been susceptible to inhibition by DRB, regardless of whether they contain RNA or DNA. The RNA viruses examined included those of influenza and polio; among the DNA type, vaccinia and herpes simplex viruses and adenovirus were studied.

A benzimidazole derivative structurally very different, 5-methyl-2-D-ribobenzimidazole, possesses the unique ability to increase the yield of influenza virus from infected tissues.[63,72] At concentrations causing increased yield of virus, 5-methyl-2-D-ribobenzimidazole did not increase the metabolic activities or cellularity of the host tissue. It had no enhancing effect on the multiplication of polio or vaccinia viruses.

2-(α-Hydroxybenzyl)-benzimidazole (HBB), the compound to be discussed at some length below, is remarkable in yet another way in that it inhibits enteroviruses but not myxo-, adeno-, arbor, pox, or reo viruses.[14] It is noteworthy that at concentrations sufficient to cause marked inhibition of enteroviruses, HBB is nontoxic for cells and does not inhibit cellular metabolic activities.*[14,26,74]

2-(α-Hydroxybenzyl)-benzimidazole (HBB). The structure of HBB, shown in Fig. 1, indicates that this compound cannot be readily conceived to be a close struc-

*I. Tamm: Unpublished results.

†A. A. Newton and I. Tamm: Unpublished results.

*A. C. Hollinshead: Personal communication.

tural analogue of any known metabolite. This structural observation is in line with the available biochemical evidence which suggests that HBB does not act as a metabolic antagonist.*[14] Furthermore, the relationship between structure and virus inhibitory activity indicates that the hydroxybenzyl grouping at carbon 2 in the imidazole ring is of fundamental importance for the selective virus inhibitory action of HBB. Structurally, it is this particular feature of HBB that makes structural analogy between this compound and purines or α-ribazole questionable.

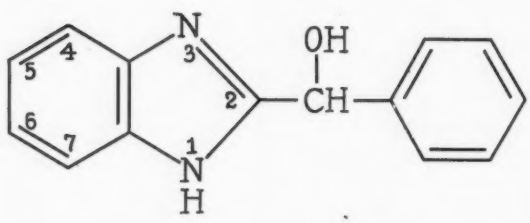

Fig. 1. Structure of 2-(α-hydroxybenzyl)-benzimidazole (HBB), a selective inhibitor of enteroviruses.

Inhibition of poliovirus by HBB. Among the numerous compounds studied by Hollinshead and Smith,[26] HBB stood out because, when fed in the diet, it protected a proportion of mice against small doses of type 2 poliovirus inoculated intraperitoneally. Observations in tissue culture indicated that HBB had an inhibitory effect on poliovirus.

Tamm and Nemes[74] found that HBB is a highly active inhibitor of the multiplication of type 2 poliovirus in monkey kidney cells in culture. HBB also reduced cell damage caused by poliovirus. The curves relating inhibition of virus multiplication or reduction in virus-induced cell damage to concentration of the compound followed an approximately parallel course. As little as 8.1 μg of HBB per milliliter sufficed to inhibit the yield of virus 75 per cent and the viral cytopathic changes 39 per cent.* At 27 μg per milliliter, 99 per cent inhibition of virus yield and 95 per cent reduction in virus-induced cell damage were obtained. Thus, at a given concentration, the inhibitory effect of HBB on virus yield was somewhat greater than that on virus-induced cell damage. In these experiments the inoculum was 500 tissue culture 50 per cent infective doses ($TCID_{50}$) of type 2 poliovirus (MEF1 strain) per monkey kidney tissue culture containing approximately 2.5×10^5 cells, and the determination of the effect of the compound was made at 48 hours.

At the concentrations indicated above, HBB did not cause microscopic changes in monkey kidney cells.

Studies of the time course of the protective effect showed that HBB delayed the development of viral cytopathic changes for several days, but did not completely prevent such changes.* Introduction of a new supply of compound during the course of the experiment did not affect the end result. The suppressive effect of the compound on viral activity was reversible, for removal of the compound was promptly followed by increased viral activity. The cell protective effect of HBB varied directly with the concentration of compound and inversely with the size of virus inoculum.

It should be emphasized that HBB had no direct inactivating effect on the infectivity of poliovirus.[74]

Experiments were done to see whether HBB would protect cells when given after infection with poliovirus had been established.* After a large dose of virus, HBB still showed some protective activity when given after the termination of the latent period, i.e., after rapid rise in new virus had begun. This indicates that HBB inhibits a relatively late step in the intracellular reproductive sequence of poliovirus and suggests that it is capable of interfering with the proper assembly of new virus particles.

*A. C. Hollinshead: Personal communication.

*I. Tamm: Unpublished results.

When given after virus had reached maximal levels, HBB was without effect on the subsequent development of maximal viral damage. This suggests that HBB has no direct effect on the cellular response to virus infection. It seems, rather, that HBB delays viral damage through inhibiting a distinct step, probably the assembly process, in virus multiplication.

The available evidence is compatible with the hypothesis that the early stages in poliovirus reproduction do not in themselves cause cytopathic changes; such changes may be the result of accumulation either of late precursor forms of virus particles or of completed virus particles.

Failure of HBB to inhibit influenza virus. Hollinshead and Smith[26] reported that HBB was ineffective against influenza virus in cultures of monkey kidney cells. This observation was confirmed by Tamm and Nemes.[74] In the chorioallatnoic membrane, Hollinshead and Smith[26] observed some inhibition of influenza A virus multiplication by HBB, whereas in the experiments of Tamm and Nemes*[74] with both influenza A and B virus, HBB had no effect on viral yield. There were significant differences in the experimental procedures employed by the two groups of workers and these differences may explain the discrepancy.

Lack of inhibition of cellular metabolic activities by HBB. At virus inhibitory concentrations, HBB did not inhibit incorporation of adenosine-8-C^{14} into RNA, incorporation of C^{14}-L-alanine into proteins, or oxygen consumption of monkey kidney cells.[14] No evidence of stimulation of these processes by HBB was obtained under the conditions employed.

Intriguing results have recently been obtained with HBB in HeLa cells.† HBB was inhibitory for a virulent and an attenuated strain of type 1 poliovirus in HeLa cells but had remarkable stimulatory effects on the cells themselves. The compound enhanced the exponential growth of HeLa cells in spinner cultures. It also increased incorporation of labeled precursors into cellular proteins and the amount of protein present per cell. In contrast, HBB did not increase incorporation of labeled precursors into nucleic acids or the amount of nucleic acids present per cell.

Virus inhibitory spectrum of HBB. Recently, studies on the viral spectrum of HBB have been extended*[14] to include all three types of poliovirus, strains of Coxsackie A and B, ECHO, para-influenza 2 and 3, mumps, adeno-, arbor B and C, vaccinia, and reo (former ECHO 10) viruses. Monkey kidney tissue cultures containing approximately 2.5×10^5 cells per culture were inoculated with 100 to 500 $TCID_{50}$ of various viruses, and development of cell damage in untreated cultures was compared to that in cultures held in the presence of HBB at 22 or 110 μg per milliliter. Cultures were examined daily for 8 days. A virus was considered to be inhibited by HBB if more than 75 per cent reduction in cell damage was observed in the presence of 22 μg per milliliter. Inhibition was considered slight if more than 75 per cent protection was observed in the presence of 110 but not 22 μg per milliliter. A virus was considered not inhibited if the development of damage followed a similar course in the presence or absence of HBB.

The results obtained are summarized in Table I. HBB appears to be a selective inhibitor of enteroviruses.

The exceptions are Coxsackie A7, Coxsackie A16, and ECHO 23. The fact that these viruses were not inhibited by HBB may provide a lead to the elucidation of ways in which these viruses differ from other enteroviruses. It should be emphasized that no instance of susceptibility to inhibition by HBB was found among viruses other than enteroviruses.

The susceptible viruses showed considerable quantitative differences in the degree of susceptibility to inhibition by HBB. With several viruses no evidence of cell

*I. Tamm: Unpublished results.
†A. C. Hollinshead: Personal communications.

*H. J. Eggers and I. Tamm: Unpublished results.

damage was seen in treated cultures throughout the course of experiments. Among such highly sensitive strains were ECHO 6 and 9 prototypes and Coxsackie B4 (Powers). With less sensitive viruses, cell changes in treated cultures became evident only after a delay ranging from a few to several days. The Mahoney strain of polio 1 and the Saukett strain of polio 3 were only slightly inhibited by HBB. However, several other strains were markedly inhibited. Comparative studies with virulent and attenuated polioviruses revealed no differences in susceptibility to HBB referable to virulence or attenuation of the strains.

In experiments in which yield of virus was determined in the presence or absence of 49 μg HBB per milliliter, it was found that the compound reduced the yield of ECHO 6, 7, and 9 prototypes and Coxsackie B4 (Powers) viruses to one-millionth or less of the yield in untreated controls. This provides additional support for the hypothesis that HBB inhibits viral cytopathogenicity by inhibiting virus multiplication.

HBB had no significant effect on yield of para-influenza 3 or vaccinia viruses. Lack of effect on multiplication of these viruses correlates with the lack of effect of HBB on cytopathic changes caused by these viruses.

It should be emphasized that HBB had no direct inactivating effect on the infectivity of viruses whose multiplication it inhibited and that its toxicity for cells was low. At 22 μg per milliliter HBB caused no detectable microscopic changes in cells observed for 8 days; at 110 μg per milliliter, minor changes developed after several days of incubation.

Table I. *Inhibition of viral cytopathogenicity by 2-(α-hydroxybenzyl)-benzimidazole (HBB) in monkey kidney cells*

Viruses inhibited by HBB	*Viruses not inhibited by HBB*
Polio 1, 2, 3	Influenza B
Coxsackie A9	Para-influenza 2 and 3
Coxsackie B1, B2, B3, B4, B5, B6	Mumps
ECHO 4, 6, 6′, 7, 9, 11,12, 19	Adeno 2, 3, 4
	Arbor B and C
	Vaccinia
	Reo (former ECHO 10)
	Coxsackie A7 and A16
	ECHO 23

Data from Eggers, H. J., and Tamm, I.: Fed. Proc. 19:407, 1960, and unpublished results.

Naturally occurring substances

Capsular polysaccharide of Klebsiella pneumoniae. Among natural products, the capsular polysaccharide of *K. pneumoniae* inhibited selectively the multiplication of pneumonia virus of mice (PVM) in the mouse lung and of mumps virus in the chicken embryo.[18,19,32] The polysaccharide had no effect on the multiplication of influenza A or B virus in the mouse or in the chick embryo. At concentrations inhibitory for PVM or mumps, the polysaccharide caused no apparent damage to the respective hosts. Furthermore the polysaccharide was capable of modifying the course of PVM pneumonia in the mouse by converting a virus infection that is rapidly fatal in control animals into a mild illness from which treated animals recovered. When polysaccharide was administered 2 to 3 days after infection, further multiplication of the virus was inhibited and soon ceased entirely. At the same time, the progress of the pneumonia was retarded and the lesions ultimately resolved. The capsular polysaccharide was effective only when given by the same intranasal route as the virus.

There are no reports in the literature concerning studies of the effects of *K. pneumoniae* polysaccharide on virus multiplication or cytopathogenicity in cells cultivated in vitro.

Products of penicillium molds. M-8450, a crude culture filtrate from *Penicillium stoloniferum*,[55,56] and helenin, an acetone precipitate of an extract obtained from the culture pellicle of *Penicillium funiculosum*,[61,62] possess selective virus inhibitory activity both in vivo and in vitro. The re-

sults of comparable experiments with the two materials indicate that the activities of M-8450 and helenin are closely similar. Recent combined chemical and biologic studies with helenin have indicated that the active principle may be ribonucleoprotein.[47] No information is available on the chemical nature of M-8450.

M-8450.

Virus inhibition in intact animals. M-8450 injected intraperitoneally 24 hours before inoculation of virus prevented the death of a proportion of mice subsequently infected with MM encephalomyelitis virus, Semliki Forest, or type 2 poliovirus by a peripheral route.[55,56] It was ineffective when given 4 to 5 hours after virus inoculation. The effectiveness of M-8450 was much reduced when the material was administered by routes other than the intraperitoneal. This substance also protected mice against ECHO 9′ (Holt) virus.[10] In chemoprophylactic experiments with type 1 poliovirus in monkeys, M-8450 given intraperitoneally prolonged the incubation period and reduced the incidence of paralysis in animals infected by the subcutaneous route.[8]

Virus inhibition in cell cultures. Pretreatment of monkey testicular cells in culture with M-8450 prevented the cytopathogenic effects of three immunologic types of poliovirus.[35] The available evidence indicates that M-8450 had no direct inactivating effect on viral infectivity[35,36]; rather, M-8450 appears to act by inhibiting the multiplication of poliovirus. In the presence of M-8450, infected cultures failed to produce detectable amounts of virus.

Protection of cells against the cytopathogenic effects of poliovirus was obtained only under rather restricted conditions. Time of administration, cell type, temperature of incubation, and medium all had an important effect on the outcome of experiments. The first two factors are briefly discussed. When large amounts of M-8450 were employed, it was necessary to pretreat cells for 5 hours before virus inoculation to bring about a protective effect. With smaller amounts, pretreatment for 24 hours was necessary. Poliovirus was inhibited by M-8450 in monkey testicular and human kidney cells but not in monkey kidney cells.* The puzzling failure of M-8450 to inhibit poliovirus in monkey kidney cells is of some practical importance because of the widespread use of such cells in experimentation with this virus.

Available information on the virus inhibitory spectrum of M-8450 in monkey testicular and human kidney cells is summarized in Table II. All three immunologic types of poliovirus were inhibited by M-8450; not inhibited were adenovirus 4, B virus, and herpes simplex virus. Certain simian viruses were inhibited by M-8450, while others were not.

In summary, M-8450 inhibits certain viruses in certain cells. It does not inhibit all viruses that grow in monkey testicular cells, neither does it inhibit in all cell systems the viruses that it inhibits in monkey testicular cells. It is of interest that M-8450 lacks antibacterial activity.[36]

Helenin.

Chemistry. In combined chemical and biologic studies,[47] helenin was extracted from the mycelium of the mold *P. funicu-*

Table II. *Inhibition of viral cytopathogenicity by M-8450*

Cells	*Viruses*	
	Inhibited	*Not inhibited*
Monkey testicular	Polio 1, 2, 3 SV 4* SV 11 SV 12	Adeno 4 B SV 1 SV 2 SV 15
Human kidney	Polio 1, 2, 3	Adeno 4 Herpes simplex

Data from Hull, R.N., and Lavelle, J.M.: Proc. Soc. Exper. Biol. & Med. 83:787-790, 1953, and Ann. New York Acad. Sc. 58:1188-1194, 1954, and from Hull, R. N.: Unpublished results.

*SV = Simian virus.

*R. N. Hull: Personal communication.

losum by homogenization in 0.005M tris-(hydroxymethyl)-aminomethane or phosphate buffer, pH 7, containing 0.005M Mg^{++}. Helenin was then precipitated by addition of one volume of acetone and taken up in more of the same buffer. About 20 Gm. of such material was obtained from an 80 gallon fermentation. All fractionation steps were carried out near 0°. The acetone-precipitated material was further purified by repeated centrifugation cycles at 100,000 g. for 2 hours. Virus inhibitory activity sedimented completely. From every gram of acetone precipitate, a 40 to 50 mg. pellet was obtained with a twenty-five–fold increase in potency. The material was active in mice at 50 to 100 μg. It contained about 40 per cent protein. This material showed a well-defined absorption peak at 260 mμ, and it gave a pentose test with orcinol and with sulfuric acid cysteine. No desoxyribose was detected. The perchloric acid hydrolysate contained guanine, adenine, cytosine, and uracil.

The purified helenin was unstable to lyophilization and to repeated freezing and thawing. Removal of Mg^{++} by dialysis caused loss of activity. Helenin was more stable when stored in 0.25M sucrose, but even under these conditions inactivation occurred.

The best preparations of helenin were heterogeneous when examined by electrophoresis and ultracentrifugation. It is likely that the inhomogeneity was caused by alteration of the native helenin and was not due to the presence of extraneous impurities. All the physical and chemical observations, including stability data, suggested that helenin is a ribonucleoprotein.

Virus inhibition in intact animals. In studies on virus inhibition in intact animals, helenin was used in the form of an acetone precipitate of an extract obtained from the culture pellicle of *P. funiculosum*. Helenin protected against death a proportion of mice infected either with Columbia SK encephalomyelitis or Semliki Forest virus.[61,62] For optimal effect, helenin had to be given within 3 hours of the time of infection. This substance also protected mice against ECHO 9′ (Holt) virus.[10] It had no effect on mortality of mice infected with swine influenza virus. In experiments on prophylaxis with type 2 poliovirus, helenin prolonged the incubation period but did not alter significantly the incidence of paralysis.[9] In monkeys, helenin given before as well as a short time after inoculation of type 1 poliovirus prolonged the incubation period and lowered the incidence of paralysis. In experiments in which administration of helenin was delayed until animals exhibited fever or paralysis, no therapeutic effect was obtained.

Virus inhibition in cell cultures. Helenin preparations inhibited the cytopathogenicity of Semliki Forest virus in chick embryo fibroblasts in vitro.* Both crude and highly purified preparations of helenin were effective. Degree of protection was proportional to concentration of helenin. In these experiments, the amount of virus inoculated per tube was usually 2×10^4 plaque-forming units (PFU), and it was sufficient to cause degeneration of all cells within 24 hours in the absence of helenin. Although observations for protection in cultures treated with helenin were commonly made 24 hours after virus inoculation, in some instances cells were observed for 3 to 5 days and it was found that protection persisted for such extended periods. As with M-8450, protection was observed only when the substance was added to cultures several hours prior to virus inoculation. Helenin added simultaneously with virus did not inhibit viral cytopathogenicity.

Some preparations of helenin which showed protection against Columbia SK encephalomyelitis virus in the mouse were not active in tissue culture against Semliki Forest virus. However, it should be emphasized that all preparations active in tissue cultures were also active in the animal assay. Correlation of activity in the two systems did not depend on purity of the preparations.

*C. O. Gitterman: Personal communication.

Induced substances from animals or cultured cells

Interferons. It is well known that viruses may interfere with the multiplication and cytopathogenicity of one another. One of the early instances of interference recognized as such, apart from specific immunity resulting from antibody, was that between a neurotropic and a viscerotropic strain of yellow fever.[16,34] The neurotropic variant, which by itself caused only transitory fever in rhesus monkeys, could effectively protect these animals against an otherwise fatal dose of viscerotropic yellow fever virus given simultaneously. Neurotropic yellow fever virus also afforded protection against the serologically distinct Rift Valley fever virus in mice. Rift Valley fever virus, which is only mildly pathogenic in monkeys, suppressed the viscerotropic yellow fever agent in monkeys.

Monkeys infected with lymphocytic choriomeningitis virus failed to become paralyzed when superinfected with the immunologically unrelated poliovirus.[12] The sparing effect of lymphocytic choriomeningitis infection was associated with an absence of detectable amounts of poliovirus in the cervical enlargement of the spinal cord of monkeys which had been infected with both viruses.

Interference between viruses has also been demonstrated in cells grown in culture. The precise mechanism of viral interference has not been clarified. It is possible that several mechanisms are operative, for the experimental observations interpreted as interference are diverse indeed. Several reviews may be consulted for both facts and theories.[21,38,58,59,80]

In studies on interference among strains of influenza A and B viruses, evidence was obtained that interference induced either by infective or heat-inactivated virus involves certain biosynthetic mechanisms of host cells.[79] It was found that chemical or physical inhibition of metabolic activities in host tissue rendered the tissue unresponsive to interfering virus. Virus which in untreated controls would interfere with the multiplication of test virus failed to have any effect on the multiplication of test virus in cultures held in the presence of 2,5-dimethylbenzimidazole or at 4° C. This finding was obtained when either active or heat-inactivated virus was used as the interfering agent. It was of particular significance that inhibition of biosynthetic processes in cells prevented the establishment of interference by heat-inactivated virus, for such virus does not reproduce, and therefore failure of such virus to interfere could not be explained on the basis that 2,5-dimethylbenzimidazole and chilling acted by merely preventing multiplication of the interfering virus. Instead, it seemed likely that a biosynthetic product of the host was implicated in interference.

Isaacs and co-workers[5,6,38,40-43,48] made the remarkable discovery that in host tissue inoculated with inactivated virus, a nonviral biologic substance is synthesized which is capable of interfering with the multiplication and also the cytopathogenicity of several viruses. This was called interferon. As originally described, interferon is produced in and released from the chorioallantoic membrane which had been inoculated with inactivated influenza virus. When transferred to normal tissue, interferon imparted a degree of resistance to infection with myxo- and vaccinia viruses.

Preparation and properties of interferons. Interferon was originally not found to be produced by incubation of live influenza virus on the chick chorion during a 24 hour incubation period.[5] However, Tyrrell[78] found that interferon was produced in tissue cultures of bovine kidney infected with influenza virus. In confirmation of Tyrrell's findings, Burke and Isaacs[6] found that interferon was produced after the first 24 hour incubation when live virus was incubated with chorioallantoic membranes over a period of 3 days.

The properties of interferons prepared and studied in different systems are closely similar[24,25,38,80] The combined evidence indicates that interferons are stable, nonviral proteins which are synthesized in suscep-

tible host cells after exposure of such cells to a variety of infective or inactivated viruses. The interferons produced are in turn active against a great variety of viruses. However, some viruses are more sensitive than others to the interfering action of interferon.

Species specificity of interferons. Interferons show species specificity. Tyrrell[78] found that interferon prepared in chick cells was much more active when tested in chick cells than when tested in calf cells, and the converse was equally true.

This observation has been confirmed by Isaacs and Westwood[44] and Porterfield,[54] who found that interferon prepared in rabbit tissue culture was effective against vaccinia virus when tested in the rabbit skin, whereas interferon prepared in chick tissue culture had only a weak effect upon the same virus in the rabbit. When the same two preparations were tested in parallel against vaccinia virus in chick cells in vitro using the plaque inhibition technique, it was found that the material prepared in rabbits was inactive, whereas the material prepared in chick tissues showed considerable inhibition zones. In the plaque inhibition test, the zone of protected cells was directly proportional to the concentration of interferons applied.

Wagner[80] used influenza A virus to produce interferons in chick embryos, duck embryos, and mice. With the Eastern equine encephalitis type as the test virus, he found a general pattern of specificity of interferons analogous to the pattern discussed above.

Lack of immunologic characteristics of interferon. Interferon appears to be nonantigenic.[38] Sera prepared in rabbits and a chicken by repeated injection of concentrated interferons did not neutralize the interfering activity of interferon.[38] The total amount of antigen may have been insufficient to induce neutralizing antibodies in demonstrable amounts. It has therefore not been possible to determine the immunologic relationship among interferons prepared in cells from different animal species.

Interferon is not neutralized by immune serum against the virus used to induce its production.[25,38,80] Likewise, hyperimmune sera with high titers of antibody against influenza virus hemagglutinin or soluble complement-fixing antigen also failed to neutralize the interfering activity of interferon prepared in chick embryos infected with influenza A virus.[80]

Virus inhibition by interferon. The virus inhibitory activity of interferon is characterized by the following features: (1) interferon does not inactivate the infective property of extracellular virus, (2) virus adsorption to host cells is not affected by interferon, (3) interferon reduces virus yield from infected cells, and (4) interferon inhibits viral cytopathic changes.

In all these respects, interferon preparations showed identical characteristics, regardless of source.[25,38,80]

Little can be said at present about the potency of interferon as a virus inhibitor, because interferon has not been obtained in pure form. In some experiments, it has been necessary to concentrate interferon-containing tissue culture media or tissue fluids, whereas in others it has been possible to dilute such materials and show activity. The effect of interferons varies directly with the concentration of interferon applied, and it also depends on the sensitivity of the assay method used. The interfering effect appears in general to be independent of the dose of test virus.[48,80] However, with large doses of vaccinia virus, complete protection was found when the dose of interferon used was given the day before virus, but not when it was given together with or after virus.[44] With smaller doses of virus, complete protection was found when the same dose of interferon was given the day before or along with challenge virus, but not when it was given the day after.

With constant doses of interferon and virus, the degree of interference increased as the period of preliminary contact of interferon with cells was prolonged.[48,80] However, beyond a 24 to 48 hour period of contact, there appeared to be no further

increase in effect. After prolonged contact of cells with interferon, the susceptibility of cells to challenge virus could not be restored by washing.[80] Ho and Enders[25] found little difference in effect when interferon was introduced 3 hours before or 3 hours after virus inoculation. (Inhibition was less when interferon was added 7 and 16 hours after virus inoculation.) Wagner[80] found also that interferons could be given after virus inoculation and a marked interfering effect obtained. This can be taken as evidence that interferon affects multiplication of virus inside cells, since most of the virus had penetrated the cells 1 hour after inoculation when interferon was introduced. It is likely that the amount of interferon that is present influences the outcome of experiments on temporal relationships.

After adsorption of interferon to cells, further incubation at 37° was necessary before interference was established.[48] Incubation at 4° did not lead to the establishment of interference. This suggests that some metabolic activity may be necessary for the interfering action of interferon to become established.

Effects of interferon on cellular metabolic activities. Interferon is considered to be of low toxicity to tissues.[44] Issacs[39] has studied the metabolic effects of interferon in primary cultures of chick embryo fibroblasts. It is of considerable interest that interferon stimulated glycolysis in cells, as measured by carbon dioxide evolution. The total lactic acid found at the end of incubation in a tris-buffered medium free of bicarbonate was two to three times greater in interferon-treated cells than in control cells.[45] The oxygen uptake of interferon-treated cells was 30 to 50 per cent greater than that of control cells.

Interferon neither stimulated nor depressed cell division.[39] Wagner[80] has preliminary data indicating that prolonged exposure to interferon does not significantly affect plating efficiency or generation time of cells and does not decrease cellular carbohydrate metabolism.

Effects of interferon in vivo. Interferon prepared in the chorioallantoic membrane and inoculated on the chick chorion in ovo inhibited the development of pocks by vaccinia virus but did not have a significant effect on pock formation by herpes simplex virus.[41] As was mentioned above, interferon prepared in rabbit kidney cells protected rabbits against intradermal infection with vaccinia.[44] Interferon prepared in the allantoic sac protected a proportion of chick embryos against death from Eastern equine encephalitis virus infection.[80] The most potent preparations of interferon gave a thirty-fold reduction of LD_{50} of challenge virus. By comparison, chick embryo cell cultures treated with interferon were resistant to 10^9 PFU of Eastern equine encephalitis virus. As Wagner has emphasized, this discrepancy could be attributed to considerable dilution of interferon in the extra-embryonic and extracellular fluids of the intact host and to the large number of cells potentially susceptible to infection in the chicken embryo.

Role of interferon in persistent infections. It has been known for some time that animal viruses can persist in tissues for long periods without producing overt manifestations of infection. More recently it has been found that cell cultures may also be persistently infected.

Henle and co-workers[3,13,22,23] have made a comprehensive study of persistency of myxoviruses in stable cell lines and of resistance of these cultures to superinfection. Persistent infections with Newcastle disease (NDV) and mumps viruses were readily established in cultures of the MCN strain of cells (derived from a leukemic patient) or in cultures of the Lung-To strain (derived from human embryonic lung). The viruses propagated generally in the absence of recognizable cytopathic effects. It should be emphasized that at most only 10 per cent of the cells could be shown to yield infectious virus and that the virus-producing cells contained not more than one infectious unit of virus per cell at any given moment.

The persistently infected cultures showed a high degree of resistance to superinfection with vesicular stomatitis virus (VSV). VSV adsorbed to resistant cells and entered into eclipse, but failed to reproduce itself. Prolonged exposure of persistently infected cultures to specific antiviral immune serum reduced the amount of myxovirus present and the degree of resistance to VSV. However, on removal of the serum after many days' treatment the myxovirus reappeared in all but one instance.

The small amount of myxovirus present in chronically infected cultures appeared insufficient to account for resistance to superinfection with VSV in terms of interference between two viruses. It is, therefore, of great interest that ultraviolet-inactivated myxovirus preparations, when added to uninfected cell cultures, led to the subsequent release into the medium of a substance which induced in fresh cells a transitory resistance to infection by VSV. The properties of this substance matched those of interferon. Even more pertinent to an understanding of the mechanism of resistance of persistently infected cells to superinfection was the finding that some interferon was detected in cultures persistently infected with mumps virus. These results clearly suggest that interferon may play an important role in the establishment and maintenance of persistent infections in the system studied by Henle and co-workers.

It should be emphasized that MCN cells persistently infected with NDV showed markedly increased aerobic glycolysis and concomitant accumulation of lactic acid, while the mumps-infected cells showed only slightly increased glycolysis.[20] Increased glycolysis was accompanied by an increase in glucose consumption. Oxygen consumption of cell cultures infected with NDV or mumps virus did not differ appreciably from that of uninfected cultures. Thus, persistently infected cultures showed metabolic alterations similar to those in uninfected cells treated with interferon.

Ho and Enders[25] have obtained evidence that the initiation and maintenance of a chronic infection with type 2 poliovirus in primary human amnion cell cultures depend at least in part upon the presence of a factor which has the properties of interferon. This factor has also been demonstrated in a chronic infection of HeLa cells, but here its role in maintaining the infection has not been definitely established.

Remarks on selective virus inhibitors

The discovery of inhibitors of virus multiplication that lack toxicity for host cells when used at virus inhibitory concentrations has opened new approaches to the study of those biochemical properties and requirements of viruses that are virus specific. The distinguishing characteristic of these selective inhibitors is that they not only inhibit the multiplication but also the cytopathic effects of viruses. Each has shown some protective activity in experimental virus infections in animals.

It is of interest that the selective virus inhibitors that have been discovered come from widely divergent sources and represent substances of strikingly diverse chemical nature. These substances include 2-(α-hydroxybenzyl)-benzimidazole (HBB), a small molecular synthetic chemical; helenin, a ribonucleoprotein derived from a penicillium mold, and M-8450, a culture filtrate; and interferon, a proteinlike substance produced in animals or cultured cells in response to virus action.

Among synthetic chemical compounds, HBB is uniquely selective in its virus inhibitory action. HBB interferes with a process that is of vital significance in the reproduction of polio, Coxsackie B, and ECHO viruses, all of which are enteroviruses, but is of no significance in the reproduction of several other groups of viruses and of no demonstrated importance to host cells. This process may involve the assembly of virus precursors into virus particles.

Susceptibility to inhibition by HBB serves as a characteristic on the basis of which enteroviruses can be distinguished from all other animal viruses which have

thus far been examined. Recently, yet another characteristic of enteroviruses has been recognized. Sulfhydryl groups of virus protein appear to be necessary for attachment of enteroviruses to erythrocytes.[53] Whether there is any link between these two characteristics is a matter for further investigation.

Studies on compounds related to HBB may lead to the development of new virus inhibitors of even greater activity and selectivity. Furthermore, knowledge of structure-activity relationships may provide a clue as to the precise mechanism of action of HBB. Earlier work with benzimidazole derivatives provided a striking demonstration of the dependence of virus inhibitory activity and toxicity on the structure of derivatives. Often the two properties varied in parallel; however, in certain instances they varied independently and thus compounds with increased selectivity were obtained. Also, knowledge of structure-activity relationships provided an early indication concerning the possible mode of action of some of the derivatives.

Shope[62] has pointed out that the active principles in helenin and M-8450 may be very closely related and may even be the same substance. M-8450 not only shows virus selectivity but is active in certain tissue culture cells and not in others. Information concerning the virus inhibitory spectrum of M-8450 and helenin is too limited to permit general conclusions.

Interferons appear to have a broad virus inhibitory spectrum in that small (polio- and encephalitis), medium (myxo-), and large (pox) viruses are all affected. However, interferon shows remarkable species specificity in that its interfering activity is much greater in cells or tissues of the animal species in which it was prepared than in cells or tissues of heterologous species. There are indications that interferons may play an important role in the natural history of virus infections by limiting the yield of virus per cell and also the spread of virus in the host organism. Interferons may be a factor in limiting the course of acute virus infections and in restricting chronic ones to inapparent infections.

The precise mode of action of HBB, M-8450, helenin, and interferons is not known. There is reason to believe that they act through different mechanisms, as there are considerable differences in their virus inhibitory spectra. None of these substances have a direct inactivating effect on the infectivity of virus particles, and there is evidence that HBB and interferons do not affect virus adsorption to host cells. The site of virus inhibitory action of HBB and interferons appears to be intracellular; that of M-8450 and helenin is not known. There is evidence that HBB interferes with a distinct step, probably the assembly process, in the reproduction of enteroviruses.

The effects of HBB and interferons do not appear to be wholly limited to the process of virus multiplication, in that each has shown stimulatory effects on certain metabolic activities of the host cells. It is not possible to decide whether these effects on host cells are, indirectly, the cause of virus inhibition or whether they merely represent concomitant phenomena.

It should be pointed out that viruses susceptible to inhibition by HBB or interferon show considerable quantitative differences in the degree of susceptibility.

It remains to be determined whether any of the selective virus inhibitors that have been discovered, or substances with similar activity that may be discovered in the future, have any application to chemoprophylaxis or therapy of virus diseases in man or animals. There can be little question that, with the aid of selective inhibitors of virus multiplication, much new, fundamental information may be gathered on viruses, their multiplication, and cytopathogenicity.

Conclusions

Antagonists of normally occurring cell metabolites are useful tools in studies on the requirements and mechanism of virus reproduction, especially in experiments designed to show the role of host cell metabolism in virus multiplication. However,

among the antagonists which have been studied, none have shown virus selectivity. Thus, studies with metabolic antagonists have not thrown light on those biochemical features of viruses which are virus specific. At virus inhibitory concentrations, antagonists have shown inhibitory effects on cellular metabolic activities leading to degenerative changes in cells. It is not surprising, therefore, that metabolic antagonists have failed to protect cells against viral damage.

Selective inhibitors of virus multiplication have been discovered among synthetic chemical compounds, mold products, and substances induced in cells by virus action. The available evidence indicates that these virus inhibitors, 2-(α-hydroxybenzyl)-benzimidazole (HBB), M-8450 and helenin (a nucleoprotein), and interferons (proteinlike substances), do not inhibit vital cellular metabolic activities when used at virus inhibitory concentrations. Thus, these substances appear not to be antagonists of normally occurring cell metabolites. In fact, at present there is no basis for classifying these substances with metabolic antagonists.

Virus inhibitory activity of HBB is limited to enteroviruses, whereas that of interferon extends to myxo-, arbor, and pox viruses as well. M-8450 and helenin appear to have limited virus inhibitory spectra. None of these substances possess virocidal activity. The precise mechanisms whereby they inhibit virus multiplication are not known at present.

HBB, M-8450, helenin, and interferons all protect cells in culture against viral damage and in addition possess some protective activity in experimental virus infections in animals. The cell protective effect appears to be due to inhibition of virus multiplication in the presence of these agents.

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Part IV. Thyroxine antagonists

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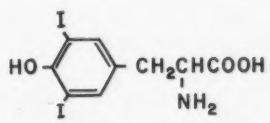
The recent revival of interest in compounds capable of inhibiting the target-cell actions of thyroxine (T_4) justifies a review of the history of such studies. It must be remembered that, with the discrediting of the early theories of vitamin action through inhibiting vague toxic effects of various substances, the possibility of hormone antagonists had to await the enunciation in the early 1940's of the broad concept of specific metabolic antagonists. In fact, the first planned attempt to discover structures with anti-T_4 activity was done by Woolley,[29] influential in establishing the antimetabolite theory. Present-day realization of the shortcomings of Woolley's early study does not rob it of its pioneering importance.

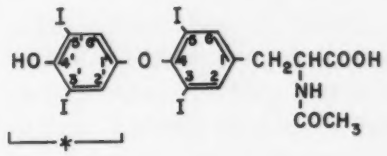
The earliest claim for T_4 antagonism by a specific chemical compound appears to have been made in 1931 by Abelin,[1] on behalf of diiodotyrosine

I 

in lowering the elevated metabolism of experimental animals. The next year, a beneficial effect of both diiodo- and dibromotyrosine was claimed in thyrotoxicosis.[2] Although repeated reports of the efficacy of these two substances are to be found in the German literature, we have been consistently unable to obtain any such action in experimental animals. Indeed, no confirmation has been recorded in this country, and these materials have not received any general acceptance in the therapy of thyrotoxicosis.

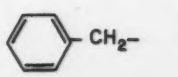
Early modifications of thyroxine structure

Woolley's[29] approach was the logical one of replacing the "outer" 4′-hydroxy-3′,5′-diiodophenyl portion* of thyroxine (shown in its N-acetyl form)

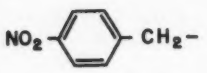
II 

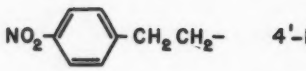
with such groups as

III $CH_3CH_2CH_2CH_2-$ BUTYL

IV  BENZYL

Original work reported in this review was supported by Research Grant A-1545 from the National Institute for Arthritis and Metabolic Diseases, U.S.P.H.S.
Received for publication March, 1960.

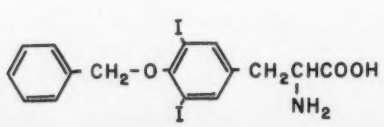
V  4'-NITROBENZYL

VI  4'-NITROPHENYLETHYL.

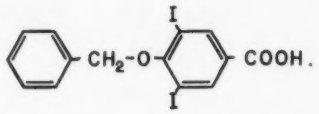
The tests used were essentially qualitative, involving inhibition of spontaneous and T_4-accelerated metamorphosis, overcoming the T_4 protection against acetonitrile poisoning in mice and antagonizing the lethal effects of T_4 overdosage in young rats. Nevertheless, it appeared from Woolley's findings with the acetylated forms that the last of the analogues shown above, the 4-(4'-nitrophenylethoxy)-3,5-diiodophenylalanine, or the 4'-nitrophenylethyl ether of 3,5-diiodotyrosine, was the most potent.

Other investigators soon picked up this lead. Williams and his associates[28] noted briefly that analogues III, IV, and VI studied by Woolley would not depress the basal metabolic rate (BMR) of normal rats but would "slightly antagonize the calorigenic action of thyroxine." The butyl ether analogue was found to be the most active, in contrast to Woolley's findings, but also the most toxic. Williams also stated that parenteral and oral administration of the 4'-nitrophenylethyl ether (VI of Woolley, above) to one thyrotoxic patient in unspecified doses was without effect. Lehman and Jorgensen[18] have very recently reported that the nonacetylated butyl ether of diiodotyrosine did not exhibit any anti-T_4 activity in the rat goiter test which will be discussed below.

Frieden and Winzler[13] soon reported successful completion of several of the syntheses started by Woolley. They found that 4-benzyloxy-3,5-diiodophenylalanine

VII 

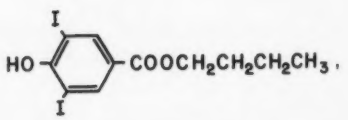
(the deacetylated IV of Woolley's series) was a more active T_4 inhibitor in the tadpole metamorphosis test than the N-acetyl form. Even more potent was 4-benzyloxy-3,5-diiodobenzoic acid

VIII 

These workers introduced the molar ratio of inhibitor to thyroxine as a rational means of judging relative inhibitory effects. Compounds VII and VIII above were calculated to yield 50 per cent inhibition at molar ratios of 37 and 8, respectively. When compound VIII was tested by Maclagan and co-workers[19] in normal mice, using a single large dose of T_4 to elevate metabolism, a molar ratio of 2,000 was required for a 53 per cent interference. Evaluated in the more sensitive thyroidectomized rat maintained metabolically on daily injections of small doses of T_4, this same compound gave 66 per cent inhibition at a molar ratio of 1,000.[4] Thus, both types of mammalian test animals were far less responsive to blockade of T_4, just as they were less responsive to T_4-like activity.

Benzoic acid derivatives

As a simplification of compound VIII, Sheahan, Wilkinson, and Maclagan[19,25] tested a series of derivatives of 3,5-diiodo-4-hydroxybenzoic acid or benzaldehyde. The most active was the butyl ester of the former

IX 

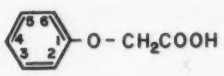
which these workers reported as inhibiting T_4 56 per cent and 82 per cent at molar ratios of 22 and 460, respectively. In thyroidectomized rats this material exerted about 40 to 50 per cent reversal of T_4 at molar ratios of both 500 and 2,000.[3]

Compound IX has had an interesting history, based on its reported lack of blocking action on triiodothyronine. The theory was advanced[20] that it might block a specific deiodination of T_4 to T_3; thus, it could prevent "activation" of T_4, but would actually enhance the effect of T_3 by also decreasing its destructive deiodination. It now seems, however, that this substance accelerates deiodination, at least of T_4 in thyroidectomized animals.[23] Further confusion is added by observations of antithyrotrophic effects.[9,10]

Incidentally, the material did not block T_4 metabolic effects in a myxedematous patient and had only as much effect on controlling human thyrotoxicosis as did the equivalent quantity of iodide.[12]

Iodinated phenoxyacetic acids

Impressed by the growth-stimulating effects produced on plants by 2,4-diiodophenoxyacetic acid, Klitgaard and co-workers[17] tested a nearly complete series of iodophenoxyacetic acids

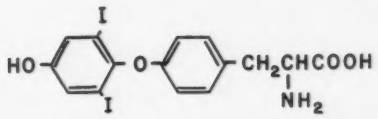
X 

synthesized by Wawzonek and Wang.[27] Again, T_4-maintained thyroidectomized rats were employed. Irregular effects were obtained at molar ratios below 300. At a ratio of 500, 50 to 80 per cent inhibition of T_4 metabolic action was produced by 3-monoiodo-, 2,4-diiodo-, 2,6-diiodo-, and 2,4,6-triiodophenoxyacetic acids.

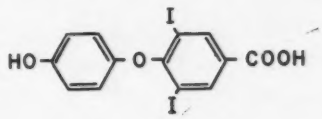
Some of these and related compounds synthesized by Barnes and collaborators[7] have been found active in the mouse test. The ethyl ester of 2,4,6-triiodophenoxyacetic acid was found about twice as active as the free acid.

2′,6′-Diiodothyronine and other analogues

Up to this time (1953), the only extensively studied T_4 antagonist with the complete thyronine structure was 2′,6′-diiodothyronine

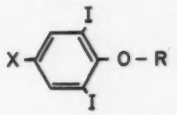
XI 

shown by Cortell[11] to reverse the antigoitrogenic action of T_4 in thiouracil-fed rats. This substance has been found by other workers to have "slight activity" against T_4 metabolic effects in mice[6] and no action in rats.[22] The qualification of slight activity was also placed[6] on 3,5-diiodothyroformic acid

XII 

in the only report of this compound.

On the basis of the iodophenoxyacetic acids and 2′,6′-diiodothyronine, it might be suggested that the configuration

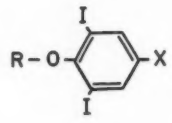
XIII 

contains a relationship adequate for T_4 inhibition. In our hands,[17] the greatest amount of activity was shown when X=I and R=CH_2COOH, yielding 2,4,6-triiodophenoxyacetic acid. It should be realized that extending this generalization far enough to include compounds III through IX would almost necessitate the paradox of including T_4 itself. Jorgensen* has suggested that it would be possible, in the instances of 2′,6′-diiodothyronine and the iodophenoxyacetic acids, to consider that the "phenolic" benzene ring occupies a

*Personal communication.

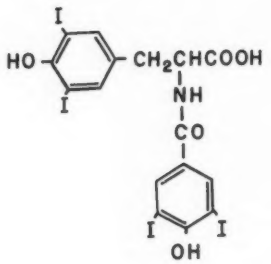
position analogous to the "amino acid" benzene ring of T_4. In this situation, the general formula XIII should be turned around:

XIII a

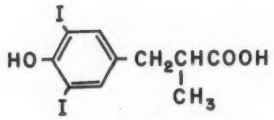


Several compounds returning more closely to the 3,5-diiodo-4-hydroxybenzoic acid type of structure have given approximately 50 per cent inhibition of T_4 maintenance of metabolism when used at molar ratios of 500 or 1,000[3]:

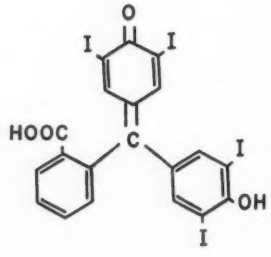
XIV



XV



XVI

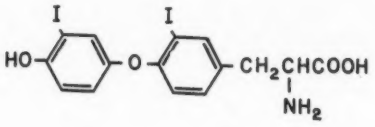


These are N-(3,5-diiodo-4-hydroxybenzoyl)-3,5-diiodotyrosine (XIV), α-methyl-β-(3,5-diiodo-4-hydroxyphenyl)-propionic acid (XV), and tetraiodophenolphthalein (XVI), which produced, respectively, 82, 61, and 55 per cent reversal of T_4 metabolism maintenance when tested at a molar ratio of 500.

Recent studies with 3,3′- and 3,3′,5′-iodothyrocompounds

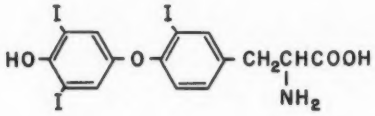
When such unusual partially iodinated thyronines as the 3,3′-diiodo (3,3′-T_2)

XVII



and 3,3′,5′-triiodo (3,3′,5′-T_3)

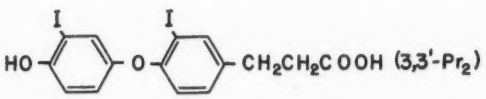
XVIII



forms recently became available,* metabolic as well as antigoitrogenic tests showed their lack of thyromimetic activity,[14,21,26] contradicting an earlier report[24] that the 3,3′-T_2 was nearly as active as T_4. The possibility that they might be capable of peripheral blockade of T_4 was then evaluated[5] in such situations as: (1) the thyroidectomized rat with metabolic rate chronically maintained with daily small injections of T_4, (2) myxedematous patients with therapeutically maintained basal metabolic rates, (3) oxygen consumption of tissues removed from injected animals, (4) T_4 reversal of thiouracil-induced goiters, and (5) in vitro T_4 analogue effects on yeast oxygen consumption.

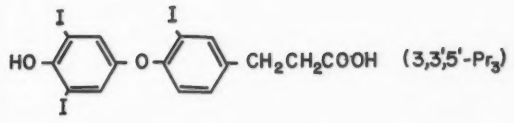
1. Thyroxine-maintained thyroidectomized rats. In addition to the thyronines shown above (XVII, XVIII), the corresponding propionic acid analogues

XIX



*Workers in this field are indebted to the generosity of Dr. R. I. Meltzer and others of the staff of the Warner-Lambert Research Institute for many of the compounds in this area.

XX

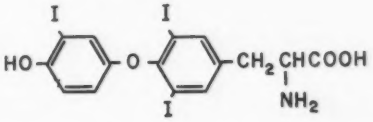


have exhibited considerable activity. Fig. 1 shows the reversal of thyroxine-maintained oxygen consumption of thyroidectomized rats (line 2) produced by the additional injection of 3,3',5'-T_3 at a molar ratio of 200:1 (line 3). After discontinuance of the 3,3',5'-T_3, thyroxine influence was restored (line 4). The suppression was again repeated, as depicted in lines 5 and 6, with essentially the same results. It is obvious that the inhibition can be quantitated by comparing the average decrease in BMR (lines 2 to 3 and 4 to 5) with the T^4-caused increases from 1 to 2, 3 to 4, and 5 to 6.

The results in Fig. 2 show that complete block of metabolic effects of T_4 was produced by 3,3',5'-T_3, 3,3'-T_2, 3,3',5'-triiodothyropropionic acid (3,3',5'-Pr_3), and 3,3'-diiodothyropropionic acid (3,3'-Pr_2), each at a molar ratio of 100 to 1 of T_4. The corresponding acetic acid analogues were about one-half as active at corresponding molar ratios.

In general, when 3,5,3'-triiodothyronine (T_3)

XXI



was used to sustain the oxygen consumption, higher ratios of inhibitors were required. The fact that definite effects were obtained is significant in view of the difference seen earlier with butyl diiodohydroxybenzoate (IX). The point of blocking with the present series of compounds appears to be similar for both T_3 and T_4.

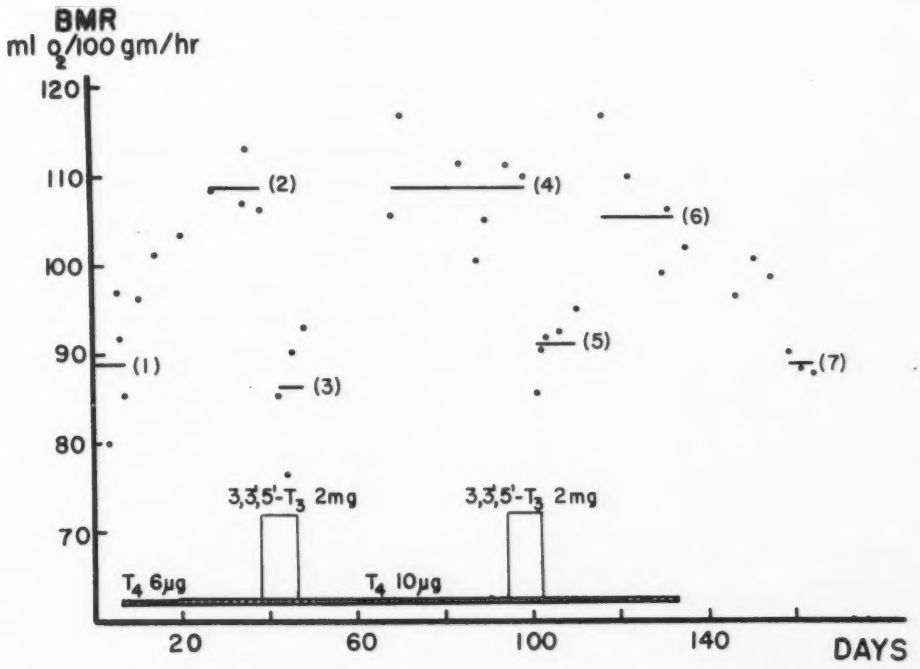


Fig. 1. BMR of thyroidectomized rats as altered by thyroxine (T_4) and 3,3',5'-triiodothyronine (3,3',5'-T_3) at dosage combinations shown. Numbered horizontal lines represent metabolic rates averaged over the time periods shown on the abscissa. Detailed explanation is in text. (From Barker, Pittman, Pittman, and Hill, Ann. New York Acad. Sc. 86:545-562, 1960.)

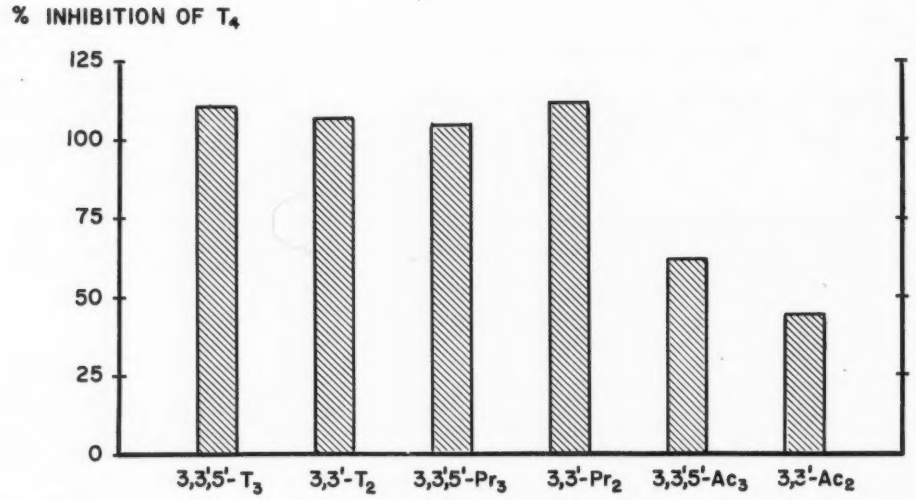


Fig. 2. Inhibition of metabolic effect of thyroxine in thyroidectomized rats. All animals received 20 μg T_4 per kilogram per day plus iodothyronine analogue at dosages yielding an analogue:T_4 molar ratio of 100:1. Compounds shown are: 3,3',5'-triiodothyronine (3,3',5'-T_3), 3,3'-diiodothyronine (3,3'-T_2), 3,3',5'-triiodothyropropionic acid (3,3',5'-Pr_3), 3,3'-diiodothyropropionic acid (3,3'-Pr_2), 3,3',5'-triiodothyroacetic acid (3,3',5'-Ac_3), 3,3'-diiodothyroacetic acid (3,3'-Ac_2).

2. Studies with myxedema in humans. Experience with human subjects revealed a much lower level of responsiveness than with the lower animals. For example, myxedematous patients maintained on desiccated thyroid or T_4 (Fig. 3) showed only 20 to 25 per cent reversal with 3,3',5'-T_3 at molar ratios of 250 to 320. Doubling the dose of antagonist produced a more marked effect, even amounting to an 87 per cent reversal in one patient receiving desiccated thyroid. One patient being treated with 3,5,3'-T_3 exhibited a 60 per cent inhibition of metabolic effect when 3,3',5'-T_3 was added at a molar ratio of 1,200:1. No depression of the elevated BMR was seen in one patient with thyrotoxicosis at 20 mg. of 3,3',5'-T_3 per day, but Benua and associates[8] have been able to cause a fall from +50 to +10, using 180 mg. per day.

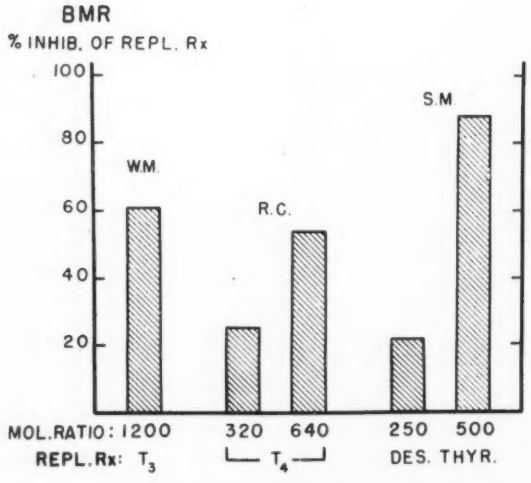


Fig. 3. Effect of 3,3',5'-T_3 on BMR of 3 myxedematous human subjects on replacement therapy *(Repl. Rx)* of 3,5,3'-T_3, T_4, or desiccated thyroid. Ordinate shows per cent inhibition of replacement therapy. (From Barker, Pittman, Pittman, and Hill, Ann. New York Acad. Sc. **86**:545-562, 1960.)

Also in contrast to the experimental work in animals no antagonistic action of 3,3',5'-Pr_3 has been obtained in man, even though higher dosages were employed than with the 3,3',5'-T_3. This suggests a much more specific importance of the side chain in the human response, a situation already noted in the case of tetra- and 3,5,3'-trisubstituted thyromimetic materials.[15]

3. *Tissue metabolism.* Thyroxine-inhibiting action of 3,3′,5′-T_3 was produced on the oxygen consumption of several tissues removed from thyroidectomized animals previously injected with T_4, as seen in Fig. 4. In this instance, the changes were less dramatic because the experimental procedure involved only a few days' injection with T_4 or T_4 plus 3,3′,5′-T_3 at higher dose levels than those used in the chronic studies, in order to meet the necessity of comparing animal with animal. Heart and liver were most effectively prevented from responding to the T_4, and skeletal muscle, represented by diaphragm, least of all.

4. *Prevention of T_4 antigoitrogenic effect.* As previously discussed, T_4-blocking activity can be quantitated in terms of ability to prevent the antigoitrogenesis of a standard dose of T_4 given to thiouracil-treated animals. Table I shows that 3,3′,5′-Pr_3 itself was without antigoitrogenic action at high dose levels. However, it was able to reverse the antigoitrogenic effect of 10 μg of T_4 32 per cent at a molar ratio of 100 and 47 per cent at 150. This is appreciable, but not as marked an action as that on metabolic rate.

5. *Yeast metabolism.* When washed, resting yeast cells *(Saccharomyces cerevisiae)*

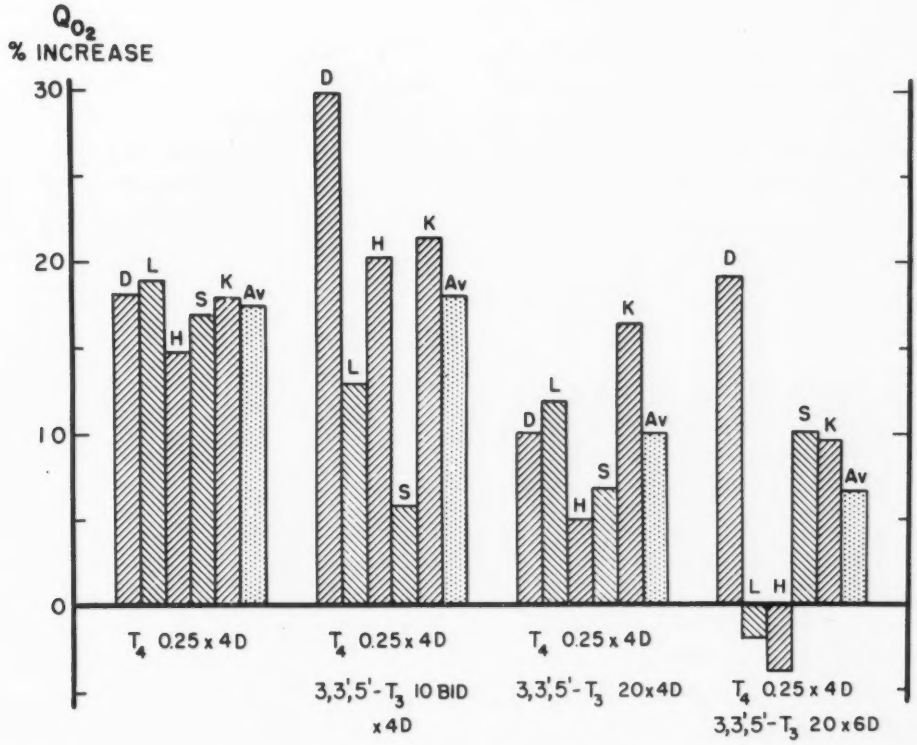


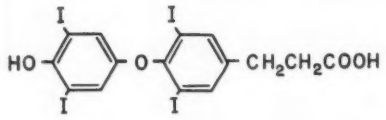
Fig. 4. Oxygen consumption of tissues from thyroidectomized rats injected with T_4 alone or in various combinations with 3,3′,5′-T_3. Values are plotted as per cent increase above control-injected animals' tissue metabolism. The vertical bars represent the following tissues, in order: diaphragm *(D)*, liver *(L)*, heart *(H)*, salivary gland *(S)*, and kidney *(K)*, plus an unweighted average (stippled and labeled *Av*). All four groups contained three animals each, all injected with 0.25 mg. T_4 per kilogram per day for 4 days and sacrificed on the fifth day. The leftmost group received T_4 only, the others, in order, 10 mg. 3,3′,5′-T_3 per kilogram twice per day for the same 4 days, 20 mg. 3,3′,5′-T_3 per kilogram once per day for 4 days, and 20 mg. 3,3′,5′-T_3 per kilogram once per day starting 2 days prior to the T_4 and continuing throughout. (From Barker, Pittman, Pittman, and Hill, Ann. New York Acad. Sc. 86:545-562, 1960.)

Table I. *Antigoitrogenic effect of thyroxine and its reversal by 3,3′,5′-Pr_3**

Treatment	*Molar Ratio*†	*Thyroid weight (mg./kg.)*	*T_4 effect (%)*
PTU 0.1% in food 11 days		200	
+ 3,3′,5′-Pr_3 1,638‡		203	
+ T_4 5‡		153	
10		120	100
15		108	
Diet without PTU		61	
PTU + T_4 10 days			
+ 3,3′,5′-Pr_3 410	50	119	101
819	100	146	68
1,229	150	158	53

*Abbreviations used: 3,3′,5′-Pr_3 for 3,3′,5′-triiodothyropropionic acid; T_4 for thyroxine; PTU for propylthiouracil.
†Molar ratio of 3,3′,5′-Pr_3:T_4.
‡All doses are given as μg per kilogram per day for the number of days shown.

were placed in a phosphate-citrate-glucose medium at 37°C., oxygen consumption fell off rapidly after one hour. Thyroxine was without influence on this, but addition of tetraiodothyropropionic acid (Pr_4)

XXII 

permitted metabolic activity to continue as long as adequate glucose was available. If 3,3′,5′-Pr_3 was added, even at the low molar ratio of 0.05, to the quantity of Pr_4 present (20 μg per milliliter), a 56 per cent depression of Pr_4 effect was obtained (Fig. 5). The inhibition became complete by a molar ratio of 0.05. This is the most striking effect we have ever seen with any of these interesting analogues. It is difficult to interpret at the present time as 3,3′,5′-Pr_3 in higher concentrations was itself able to drop oxygen consumption below the control level. Considerable further exploration is necessary.

Discussion. A few general remarks on the treatment of thyrotoxicosis seem appropriate before leaving these compounds of great contemporary interest. Considering the high doses required and the fact that in man a fatty acid side chain confers much less activity than the amino acid alanine, which is far more difficult to synthesize, it is unlikely that these compounds will replace the now well-established therapeutic procedures for suppression of the overactive thyroid gland itself, i.e., the use of substituted thioureas

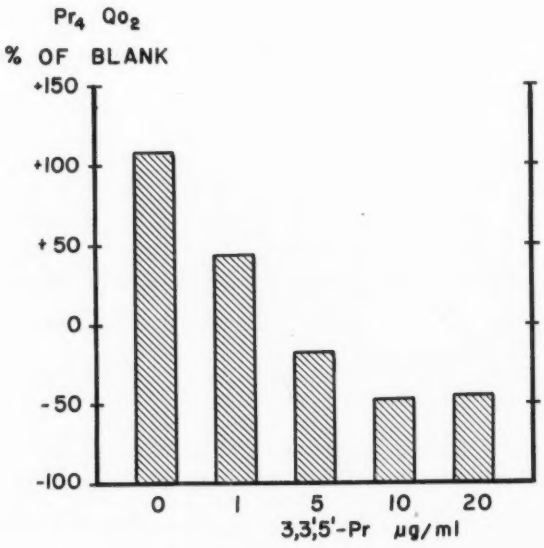


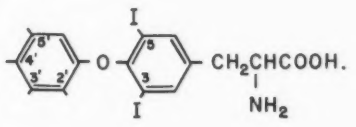
Fig. 5. Inhibition of tetraiodothyropropionic acid (Pr_4) enhancement of yeast oxygen consumption. Ordinate shows per cent change from appropriate control solution when 3,3′,5′-Pr_3 was present at the concentration shown along the abscissa.

or subtotal elimination of the thyroid gland by operation or radioiodide.

However, the mere fact of the demonstration of T_4 inhibition by compounds so closely related structurally to T_4 is in itself a stimulating observation. Furthermore, the potency of these substances in mammals is far greater than that of any previously studied by a factor of 5.

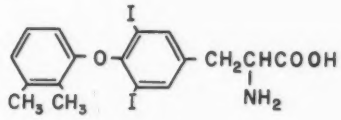
A deviation from this general point of view is being followed by Jorgensen and Kaul,[16] who have synthesized a series of 3,5-diiodothyronines devoid of the phenolic (4′) hydroxyl

XXIII



The parent compound (4′-deoxy-3,5-diiodothyronine) can also be considered as 4-phenoxy-3,5-diiodophenylalanine. In the case of 4-(2′,3′-dimethylphenoxy)-3,5-diiodophenylalanine

XXIV



these workers have the first substituted 4′-deoxythyronine exhibiting T_4-like activity.

In contrast, significant T_4-inhibiting action in the antigoitrogenesis test was found for the 2′,4′-dimethyl-, 2′,5′-dimethyl-, 2′-isopropyl-, and 2′-isopropyl-5′-methyl-substituted derivatives of compound XXIII. These materials were all unable to raise the oxygen consumption when injected alone into rats. They are at present being evaluated for anti-T_4 metabolic action. If they prove active antagonists in this test, they may act by being bound at both the 2′ and diiodotyrosine portions while the 3,3′,5′-trisubstituted thyronines can be bound at three other specific positions.

Summary

Work of the past year on antagonists of thyroxine, 3,5,3′-triiodothyronine and thyromimetic analogues has been surveyed against the background of earlier work. The studies of 1946 to 1953 concentrated on iodophenoxyacetic acids and variously substituted esters and ethers of diiodohydroxybenzoic acid or diiodotyrosine. For the most part, these required a molar ratio of about 500 of inhibitor to 1 of thyroxine for inhibition when mammalian preparations were employed.

The 3,3′-diiodo- and 3,3′,5′-triiodothyro- compounds of current interest exhibit T_4-antagonistic effects at molar ratios of 50:1 to 100:1. This suggests a more specific site of competition with T_4 than is shown by the substances examined earlier. The 2′-, 2′,4′-, and 2′,5′-substituted 4′-deoxy-3,5-diiodothyronines deserve considerably more attention since they also give promise of being antagonistic to T_4.

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(To be continued)

Medical-legal note of interest to clinical investigators

That a new and, so far, unprecedented hazard may face the clinical investigator who is not especially circumspect was brought out by the recent decision in the Municipal Court of New York in the case of Fisher vs. Thompson Medical Co.

Thompson Medical claimed that Fisher, a licensed physician practicing in the City of New York, had contracted to evaluate the appetite-controlling and tobacco habit–relieving properties of its product, Slim-Mint, along the usual lines of a well-controlled scientific investigation. Thompson Medical was to support the research with a grant of $5,000.

Thompson Medical claims that the Fisher report was unacceptable. *(It was negative.—Ed.)* In its suit for the return of the portion of the grant already paid Fisher, Thompson Medical represented that Fisher had failed to perform the research agreed on, to maintain the records, or to write a proper paper pursuant to the agreement and that the research was not conducted on a scientific basis. A statistician, testifying as an expert for Thompson Medical, stated that the data supplied by Fisher were such that no valid conclusion could be drawn from them.

The judge, the Hon. George Starke, found that those case reports actually turned over had no scientific value or significance and did not meet the accepted and recognized standards of the medical profession, that the records were slipshod, haphazard, confusing, ambiguous, faulty in completion, and unscientific. The Court (jury waived) ruled in favor of Thompson Medical and made an award of $3,000 plus interest and costs.

The implications of the case and its outcome are clear enough. It also seems too obvious to require mention that contractual arrangements are sometimes beyond the clinical investigator and that he cannot always safely assume that he does not need the help of an attorney when he enters into an agreement to conduct a scientific investigation.

Book reviews

Fluorine and Dental Health; The Pharmacology and Toxicology of Fluorine, edited by Joseph C. Muhler and Maynard K. Hine. Bloomington, Ind., 1949, Indiana University Press. 216 pages.

The battle is by no means over yet. Mention fluorine and you are bound to find a vociferous minority of angry citizens holding protest meetings against "adulteration" of the water supply. This preoccupation with what is "natural" is an interesting human phenomenon; many people will take their daily dose of Parker's Little Liver Pills in order to force "natural" *daily* bowel movements rather than believe that one bowel action in 3 days may be natural.

The editors of this book are biased in favor of communal fluoridation to decrease the incidence of dental cavities and say so in the preface: "The chief reason for holding this symposium at this time, as well as for choosing the particular topics presented, was that fluoridation is being seriously hampered because of incomplete dissemination of the scientific evidence which is available indicating the overwhelming safety of this public health procedure." Chapters by different authors are then presented on various aspects of fluorides, dealing mainly with the pharmacology and especially the safety of fluoridation.

A historical review could with benefit have been added to this book; as it is, one is suddenly pitched into Chapter 1: "Fluoride Toxicity." Even in a large textbook an introductory paragraph or two puts the whole subject into perspective and makes for more interesting reading; it is almost essential in a monograph.

During the years of the second world war, Spira, working in Great Britain, examined thousands of military personnel and found a high incidence of mottling of the teeth, as well as "mottled nails," constipation, neuralgia, paresthesiae, and other minor complaints. This has been strongly criticized by Drs. Smith and Hodge, the authors of the chapter on fluoride toxicity, and justly so; one has only to read Spira's papers to realize that his work was unscientific. For example, mottling was taken to mean fluorosis, whereas it is well known that even people living in low fluoride areas may have "white spots" on the teeth. Most damning is the fact that he used no controls: one can imagine that a high percentage of any population will have symptoms of the "Spira syndrome."

Even though Spira's work may not amount to much, he is entitled to mention in the bibliography, yet no reference to his papers could be found.

Very strong arguments, backed by recent experimental and epidemiologic findings, are presented to refute the possibility of danger from communal fluoridation. There appears to be no danger that fluoride in the usual concentration of one part per million can cause enzyme inhibition, increase gingivitis activity, or have a deleterious effect on vitamin metabolism.

Animal and human research is carefully described and analyzed, but work must still be done on various aspects of fluoride metabolism, e.g., on the relationship of high fat diet to fluoride storage: there is evidence that a high fat diet increases the tissue storage of fluoride in animals. Fluoride may be ingested from sources other than a fluoridated water supply, e.g., from tea and seafood, and thereby the safe total daily intake over a long period may be exceeded; although a remote possibility, this should be investigated in view of the above-mentioned relationship between fluoride and high fat intake.

The strongest argument for fluoridation of communal water supplies is derived from numerous large-scale epidemiologic studies which have indicated the safety of this procedure beyond any doubt. As an expensive alternative, the dentist may apply sodium fluoride to the teeth after thorough cleaning on a number of occasions at set intervals.

The legal aspect is also discussed. Thus far there has been only one adverse finding, which was subsequently reversed by a higher court. The fact that the courts of ten states in the United States have held that the fluoridation of public water supplies does not infringe upon the constitutional or legal rights of the individual is an additional powerful argument for those who advocate fluoridation.

This is essentially a book for the specialist. Anyone interested in the pharmacology of fluorine, in its relationship to dental health, and particularly in recent research work on it will find much to interest him.

For a short survey of the subject, "Medical and Biological Aspects of Fluoridation," written in traditional *Lancet* style, may be recommended (Lancet **2**:425-428, 1960).

Edel Berman

Textbook of Pharmaceutical Chemistry, seventh edition, by J. E. Driver. London, 1960, Oxford University Press. 728 pages. $14.50.

The seventh edition of *Textbook of Pharmaceutical Chemistry* by J. E. Driver is intended as a textbook for students as well as a reference work for pharmacists and chemists. A large amount of information on the recent developments in pharmaceutical chemistry has been included, most of which concerns the rapidly expanding field of organic medicinal compounds. The work is divided into three sections: the first deals with analytic methods of interest to the pharmaceutical chemist; the second considers general chemical theory and the chemistry of those inorganic materials of medicinal importance; the third takes up the chemistry of organic compounds of particular interest in the field of pharmaceutical chemistry.

In the first part there is a thorough discussion of the determination of various physical constants. Some of the essentials of the usual gravimetric and volumetric analytic procedures, together with a discussion of indicators, are given. Photometric methods of analysis, spectrophotometry, fluorometry, and the measurement of radioactivity are discussed rather briefly.

In the second part the fundamentals of modern atomic theory are presented in a concise manner, including a description of the types of chemical bonding and a short section on molecular orbitals.

The third and longest section of the work deals with organic chemistry: organic formulas, nomenclature, isomerism, analytic methods, and a systematic discussion of the general classes of organic compounds are presented. This is complete enough to give a person unacquainted with the subject a substantial grasp of basic organic chemistry and to enable him to understand the material on the synthesis of pharmaceutically important compounds.

The chemistry of glycosides, barbiturates, hormones, vitamins, proteins, antibiotics, medicinally important heterocyclic compounds, sulfonamides, and alkaloids is discussed in some detail.

Edmund J. Gaughan

Medicinal Chemistry, second edition, edited by A. Burger. New York, 1960, Interscience Publishers, Inc. 1243 pages. $37.50.

Because the second edition of *Medicinal Chemistry* represents an enormous mass of detail on an important subject which can nowhere else be found put together in such a well-designed format, it is unfortunate that the large price for this large book may be a deterrent to those who are not likely to require the very frequent use of such a reference book. Its information on structure as well as many other features of the chemistry of a fairly up-to-date list of new and a reasonably complete list of old drugs is easy to find. These make the book invaluable for those who have to deal with the chemistry of drugs, and for these Dr. Burger and his collaborators are to be congratulated.

In reviewing this book as a clinical pharmacologist rather than a chemist (which I am not), I cannot pretend to have scrutinized the chemical data critcally for, should errors exist, I would not be able to detect any but the most glaring. Fortunately, the authority of Dr. Burger and his eminent associates as chemists is unassailable. I was impressed with the breadth of the view as well as with the fullness of the detail.

The fault I find, however, is not with the chemistry; it is that the book wanders and tends to discuss, sometimes badly, subjects close to but not really on the mark. When a book becomes as big and expensive as this one, it is surely the editor's function to spend as much effort in pruning as in harvesting. This, unfortunately, was not done in preparing this edition. As information accumulates and books on even the most highly specialized subjects become bulkier and more difficult to handle, I find myself wishing that writers would limit themselves to their own specialties and omit discourses (which tend to be poor and often even foolish) on subjects outside their domain. Inevitably, if one needs information outside the specialty of the author, he will have to look for it elsewhere. Therefore these digressions can serve no useful purpose. Because *Medicinal Chemistry* has outstanding merits, I hope the authors will see my point and forgive me if I use this book as a whipping boy for all the others which have become too large, too expensive, too difficult to handle, and, for those who try to build a library, wasteful of valuable space.

In this book the sections on nonchemical material, if not cut to the bone, should have been omitted entirely. What need in a text on medicinal chemistry for a section on "The Response of Cells and Tissues to Drugs," or on "The Development, Anatomy and Physiology of the Nervous System," or on "Pain and Pain Relief," or on "Convulsions and Epilepsy"? Many of the excursions into pharmacology and clinical medicine by nonpharmacologists and nonclinicians, besides being inadequate, border on error. There is a section on "Anti-Ulcer Drugs." Admittedly this is a clinical designation which few clinicians would dare use; but why a clinical category at all? And if "Anti-Ulcer Drugs" means anything,

surely the antacids are the most important of the group; yet these were either overlooked or unrecognized as of clinical (and, I suppose, chemical) importance. There is a chapter with the strange title—"Drugs Eliciting Gastrointestinal Symptoms." Appetite stimulation is rarely thought of as a *symptom,* yet "Appetite Stimulants" is a heading in this chapter. Here a central stimulant action to increase appetite is ascribed to strychnine. For at least 25 years strychnine has been abandoned as any kind of a stimulant in clinical medicine, while modern pharmacologists view it as a drug which may produce its effects by *depression* of central inhibitory mechanisms. A sorry pharmacologic statement on the action of anorexiants compounds confusion because the author does not recognize that these drugs are central stimulants and not depressants of the appetite center.

As it stands, the chemistry in this work is invaluable for all interested parties, chemists, pharmacologists, clinicians, but the pharmacology will be an irritant to pharmacologists and inadequate for others and the clinical aspects will be useless to all. It seems to me that had Dr. Burger and associates stuck to their knitting, they would have had a more compact and less expensive book and there would have been no soft spots in this otherwise excellent work on medicinal chemistry.

Walter Modell

The Search for New Antibiotics, by G. F. Gause. New Haven, 1960, Yale University Press. 97 pages. $4.75.

The Search for New Antibiotics, by G. F. Gause, Director of the Institute of Antibiotics of the Academy of Medical Science of Moscow, is the second of a series of monographs, *Trends in Science,* published by the Yale University Press in cooperation with Yale University and the Yale chapter of Sigma Xi.

The monograph consists of three chapters. The second, "The Importance of Early Classification of Micro-organisms Producing Antibiotics," is fortunately short, for it is both obvious and dull. The first chapter, "The Distribution of Micro-organisms Producing Antibiotics," is much longer and of some practical importance and interest. The third chapter, "Micro-organisms and Cancer Research," presents evidence for the interesting possibility that mutant microorganisms with respiratory mechanisms impaired by carcinogenic agents are, in fact, analogous to malignant mutant animal cells and may therefore be used in the study of malignant processes as well as in screening operations in the search for antibiotics for cancer.

The presentations are simple and nontechnical, the bibliographies modest.

Walter Modell

The Clinical Use of Aldosterone Antagonists, by Frederic C. Bartter. Springfield, Ill., 1960, Charles C Thomas, Publisher. 211 pages. $5.

This portable symposium on such a lively and timely a topic as aldosterone antagonists could not possibly be interesting because it is so badly dated. The talks on this active subject were delivered in 1958 and, as a result, much of the material has already appeared elsewhere, even in other symposia.

The book also suffers from lack of substance; only a few papers, like the one on "The Pharmacology of Diuretic Agents and Electrolyte Problems Encountered With Their Use," by J. H. Laragh, are first rate. The discussion is uninspired.

Walter Modell

Books received

Brazier, M. A. B., Editor: The Central Nervous System and Behavior, New York, 1959, Josiah Macy, Jr. Foundation. 358 pages. $4.75.

Gause, G. F.: The Search for New Antibiotics, New Haven, 1960, Yale University Press. 97 pages. $4.75.

Luck, J. M., Editor: Annual Review of Biochemistry, vol. 29, Palo Alto, 1959, Annual Reviews, Inc. 786 pages. $7.00.

Muhler, J. C., and Hine, M. K., Editors: Fluorine and Dental Health: The Pharmacology and Toxicology of Fluorine, Bloomington, 1959, Indiana University Press. 216 pages.

Wilson, R. C.: Drugs and Pharmacy in the Life of Georgia, Athens, Ga., 1959, University of Georgia Press. 443 pages. $6.00.

Wolstenholme, G. E. W., and O'Connor, C. M.: Cellular Aspects of Immunity (Ciba Foundation Symposium), Boston, 1960, Little, Brown & Company. 495 pages. $10.50.

Note to subscribers

In having your bound volumes prepared, please instruct your binder to include the "Current Drug Therapy" articles as a part of the text pages. This is a simple binding procedure which should require no extra charge.

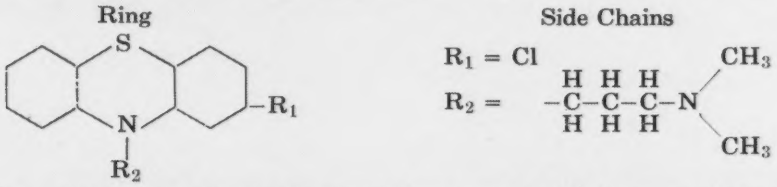
The phenothiazines

One of the most versatile series of compounds has certainly been derived from the phenothiazine molecule. This substance has now yielded at least 12 agents widely used in medicine. It is, therefore, appropriate to consider briefly some of their essential chemistry, pharmacology, and their clinical uses.

The first phenothiazine found to be of any general use in medicine was promethazine (Phenergan) which was introduced as an antihistamine. Although promethazine proved to be a very useful antihistamine, it had a serious drawback in that it caused considerable sedation, especially when the dose was increased beyond the 12.5 mg. tablet recommended for the antihistaminic effect.

It was not, however, until the development and release of chlorpromazine (Thorazine) that the phenothiazines began to assume an important role in medicine. Chlorpromazine was soon found to exert many useful therapeutic actions and rapidly became one of the most widely used drugs. Since chlorpromazine was the first potent and widely used phenothiazine and because knowledge gained from it applies, in varying degrees, to the subsequently developed phenothiazine derivatives, it is appropriate to discuss it briefly and its role in medicine, in order that we can better understand subsequent developments in the phenothiazine field.

First, it is important to consider the chemical structure of the chlorpromazine molecule, since all new derivatives are modifications of it.



So far, all important derivatives have resulted from variations in R_1 and R_2 substitutions. These substitutions have changed considerably the potency and action of the derivatives as compared to those of chlorpromazine. These new substances will be considered individually later.

Second, it is necessary to understand the pharmacology of chlorpromazine as a basis for comparison when new preparations are introduced. As yet, the exact mechanism of action of the drug is not known. It selectively inhibits the chemoreceptor trigger zone, the hypothalamus, and perhaps the reticular substance. A weak anticholinergic, antihistaminic, antispasmodic and hypotensive action is observed. Narcotic analgesic drugs and certain barbiturates are potentiated, although there is no interference with their metabolism. The pressor response of epinephrine and norepinephrine is reduced and, indeed, may be reversed for the latter. Animals receiving it show reduced hostility, certain learned conditioned responses in rats are interfered with, and fighting fish do not fight. Indeed, most overactive animal behavior is reduced. Chlorpromazine's ability to depress cells in the chemoreceptor trigger area and, to a mild degree, cells in the emetic center makes it a good antiemetic agent. Body temperature is altered. By its action on the diencephalon, it interferes with heat conservation, while its peripheral vaso-

dilatory effects lead to increased heat loss. The coronary arteries are dilated and cardiac irritability reduced.

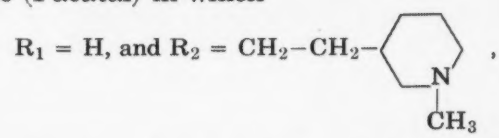
The drug is readily absorbed from the gastrointestinal tract, when given by mouth or suppository. It can also be given intravenously and intramuscularly. After administration, its action is prompt; when given orally, it begins within one half hour and reaches a peak in one hour with its therapeutic effect being maintained for approximately 4 hours. Higher concentrations are found in the brain than in other organs. It is metabolized in the body and only slight amounts are found in the urine. Approximately 15 per cent appears as the sulfoxide in the urine.

Such a versatile drug soon found many uses in clinical medicine. It was first used in psychiatry to reduce agitation, tension, anxiety, and abnormal emotional and mental states, where it had tremendous success. Next, it became widely used as an antiemetic, effective against nausea and vomiting. Later, it was found to be useful against hiccough, pruritus, agitation from withdrawal of alcohol and other drugs, and attacks of acute and chronic porphyria, and as a potentiator of anesthetics, barbiturates, and narcotic analgesic drugs. It was found to be relatively ineffective as a motion sickness remedy.

Unfortunately, its wide use soon uncovered certain toxic actions. In approximately 2 per cent of patients, an obstructive type of jaundice appeared which usually cleared promptly once the drug was discontinued. Agranulocytosis, although rare, appeared often enough to be troublesome (approximately 1 case in 50,000 to 100,000). Skin rashes, light sensitivity, and pruritus were also observed. In a rare case, gynecomastia and even lactation occurred. If the dose was large, extrapyramidal nervous system activity, varying from mild loss of skilled muscle movements to frank Parkinsonism-like activity, was produced.

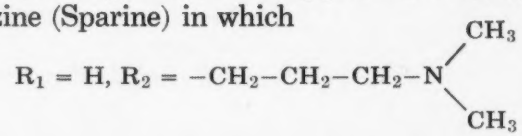
Since jaundice, agranulocytosis, and other toxic effects had not been observed to any extent with promethazine, it was thought by many that the absence of a chlorine atom on the phenothiazine ring and of a propyl side chain might be factors in reducing its toxicity.

Soon, mepazine (Pacatal) in which



was introduced, but found to be a weaker, much less effective drug, which, unfortunately, was still capable of causing jaundice and agranulocytosis.

Next, promazine (Sparine) in which

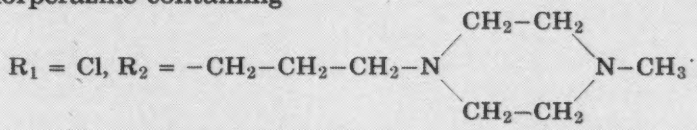


was introduced, a compound differing from chlorpromazine only in the absence of a chlorine atom on the phenothiazine ring. This compound, although highly useful, is weaker than chlorpromazine and, unfortunately, is still capable of causing jaundice and agranulocytosis, although to a much lesser degree. There were, furthermore, no special merits, other than the fact that, being a weaker drug, it could be used in combination with narcotics, barbiturates, anesthetics, and other drugs with less chance of serious reactions.

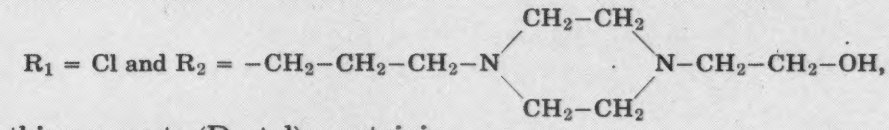
As a result of these weaker drugs, without chlorine in R_1 and with the same or different side chains in R_2 position, still exhibiting toxic properties, some came to the conclusion that it might be better to enhance potency and thus introduce less phenothiazine into the organism in the hope of reducing toxicity.

In the search for a more potent compound, a series of substitutions were tried in the R_2 position. One of the earliest of these was prochlorperazine (Compazine). This substance, while retaining the chlorine in the R_1 position had a piperazine ring introduced into the side chain. It had been observed for some time that the piperazine ring added to certain antihistamines markedly improved their ability to suppress motion sickness and increased their potency.

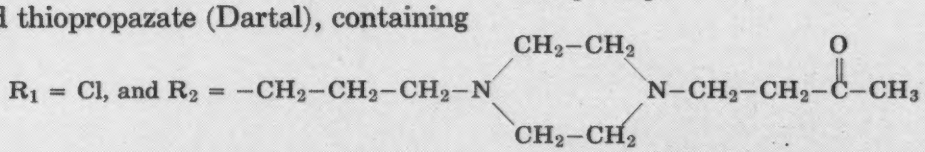
Prochlorperazine containing



was indeed a more potent drug, five times as potent as chlorpromazine. It soon gained and still has widespread acceptance in medicine. Soon after prochlorperazine was introduced, perphenazine (Trilafon) containing

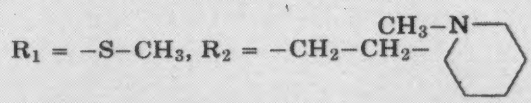


and thiopropazate (Dartal), containing



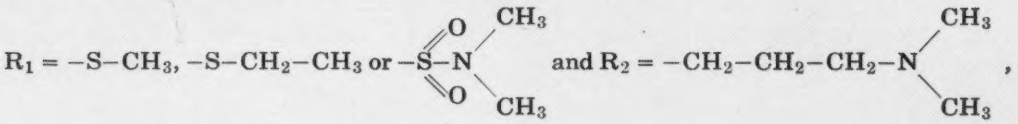
appeared. These, like prochlorperazine, are excellent drugs of the same order of potency as prochlorperazine. All three are effective and can be used wherever chlorpromazine would be indicated in therapy. They, fortunately, are much less toxic, only a rare case of jaundice being reported, and if agranulocytosis has been observed it must be exceedingly rare as no case has been reported to my knowledge. Unquestionably, these drugs represent a distinct advance in phenothiazine therapy. It was soon observed, however, that severe extrapyramidal activity occurred in a small percentage of cases, especially with perphenazine and prochlorperazine. The phenomenon, consisting of repetitive jerking, twisting, or rotation of the head, eyes, arms, legs, or trunk, appeared in some patients and especially in children, after even comparatively small doses. Although temporary, the reaction is most distressing to the patient and exceedingly frightening to observers. Furthermore, the reaction is unpredictable and does not respond well to drugs used for Parkinson's disease or other measures. No deaths have been reported, but the reaction has led to great caution in the use of these drugs in children and has stimulated search for other agents which do not possess this property.

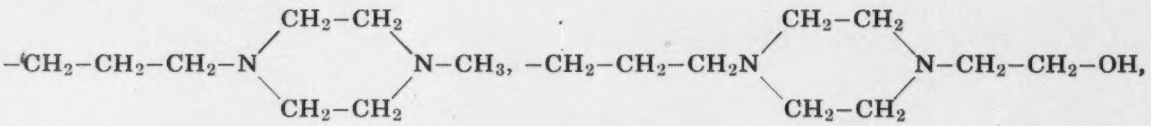
The first successful agent introduced into medicine which did not possess the ability to produce extrapyramidal overactivity was thioridazine (Mellaril), in which

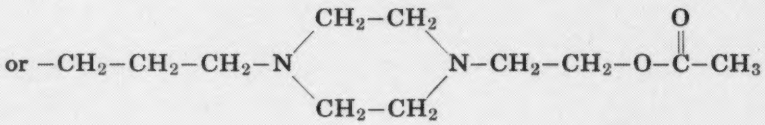


This preparation is a weak phenothiazine in the order of potency of mepazine and it exerts limited antiemetic effects. It does not, however, induce extrapyramidal activity. As yet, no jaundice or agranulocytosis has been seen, but that possibility must be borne in mind since the total dose of phenothiazine administered for therapeutic effect with this drug is in the same order of that needed with mepazine.

Other thio-substituted agents are under study, in which

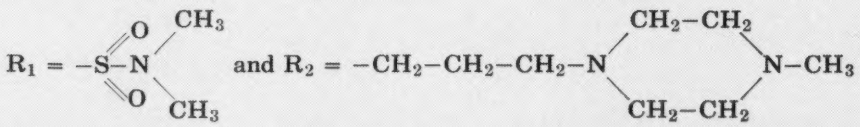






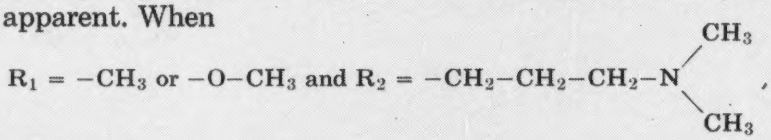
Some of these will undoubtedly prove to be not only potent, but also free from serious toxic effects.

One of these, thioperazine (Vontil),

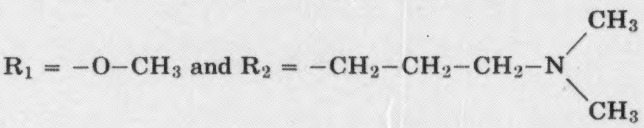


has already been found to be one of the most potent antiemetics available. It is approximately 50 times as potent as chlorpromazine as an antiemetic. It does not, however, have significant tranquilizing action. Unfortunately, it still exhibits extrapyramidal action, although not to a great degree. Since the dose needed is so small, it is reasonable to predict that there will be very little in the way of toxic effects observed. This has been the case in experimental studies to date.

The ability of fluorine to enhance potency has been observed in several chemical biologic systems and again in the phenothiazines this property becomes apparent. When

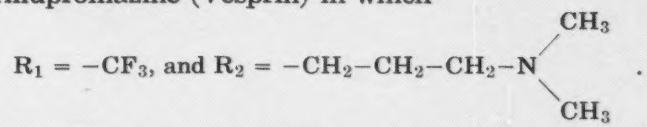


compounds are produced which have low potency, in the order of that seen with mepazine or promazine. Methoxypromazine (Tentone), in which



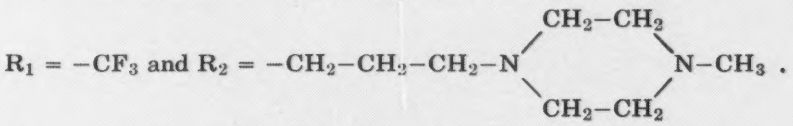
is an example of this type of agent which has recently been introduced. It is a useful, mild drug which, because of the total amount of phenothiazine introduced, may well exhibit toxic properties in the order of those observed with mepazine and promazine.

However, when the methyl group is fluoridinated, a highly potent phenothiazine results. The first of these fluoridinated preparations that appeared was triflupromazine (Vesprin) in which



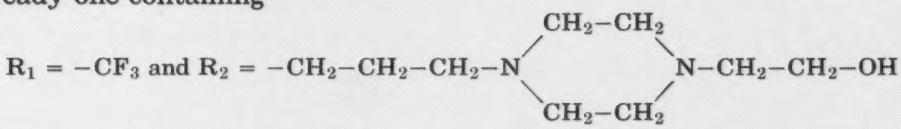
This phenothiazine has a potency between that of chlorpromazine and prochlorperazine. It is a useful agent, but again requires a larger amount of phenothiazine for satisfactory action, and since it is not as potent as prochlorperazine, it has not been used as widely.

In an attempt to improve on the phenothiazine molecule along these lines there next appeared trifluoperazine (Stelazine) in which

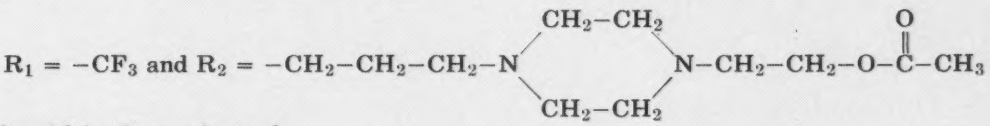


This is a highly potent phenothiazine, about 10 to 12 times as potent as chlorpromazine. It primarily exerts a tranquilizing effect. In some mentally disturbed patients, it is easily the most effective agent. Its action is longer than that of chlorpromazine. Frequently a dose every 12 hours is satisfactory. It also causes frequent and severe extrapyramidal attacks.

Unquestionably, other trifluro substituted phenothiazines will appear. Already one containing

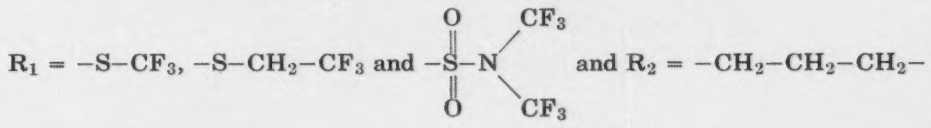


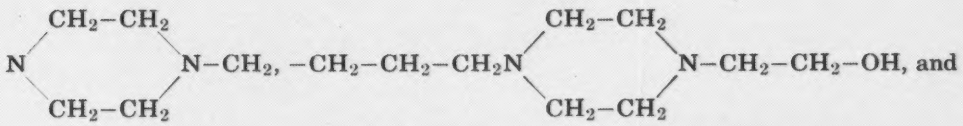
is under study and undoubtedly a molecule containing

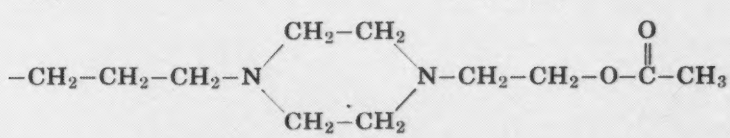


should be investigated.

On the basis of what is already known about the phenothiazines, it is not too unrealistic to suggest that molecules containing

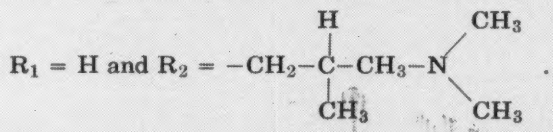






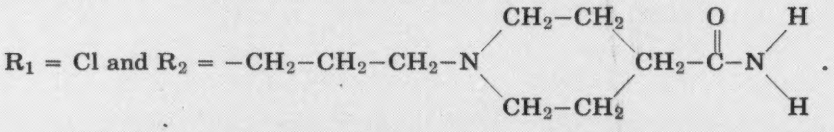
would offer exciting opportunities to reduce toxicity and increase potency in this most interesting field.

Finally, a word should be said about certain special phenothiazines. The ability of chlorpromazine to alleviate pruritus led to a study of several phenothiazines in the hope that one with enhanced antipruritic action could be developed. The first and, as yet, only phenothiazine introduced as an antipruritic is trimeprazine (Temaril),



This agent is effective and in certain situations, such as the intense pruritus exhibited in jaundiced patients with biliary tract obstruction, it is the only really effective agent. Unfortunately, it is capable of producing jaundice and agranulocytosis, although these toxic effects are exceedingly rare. Another difficulty with trimeprazine is adjustment of dosage, with a range of from 2.5 to 25 mg. 3 or 4 times daily. There is need for further search for antipruritics among the phenothiazines.

The most recent addition to the phenothiazines is pipamazine (Mornidine) which was designed primarily as an antiemetic, in which



Experimental work and clinical observations to date indicate that this phenothiazine is a weak tranquilizer, but an effective antiemetic with exceedingly low toxicity, and does not exhibit extrapyramidal activity unless the dose is 10 to 20 times the recommended dose of 5 mg. every 4 to 6 hours. It is too early, as yet, to know whether this optimistic beginning will be maintained.

Unquestionably, there will be more phenothiazines introduced in the next few months and it seems to me that three things should be considered in this field: First, is liver and blood toxicity lower than that which has been observed for potent phenothiazines, such as prochlorperazine, perphenazine, and thiopropazate? A clue may be obtained by the dose of the drug required, since smaller doses, such as 5 mg. 3 or 4 times daily have resulted in much less toxicity of this type. Second, is there less extrapyramidal activity produced, even when the dose is pushed to 10 to 20 times that recommended? Finally, does the new phenothiazine have unique properties, such as tranquilizing action, alone or with antiemetic action, mainly antiemetic action in scope, or a more selective antipruritic action?

DALE G. FRIEND, M.D.

Senior Associate in Medicine, Peter Bent Brigham Hospital
Assistant Professor of Medicine, Harvard Medical School

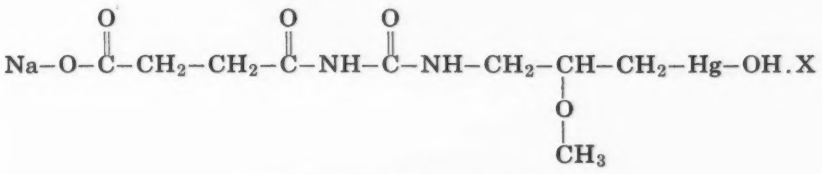
Modern diuretics and diuretic therapy

In the past decade considerable improvement in diuretics and diuretic therapy has occurred. At the beginning of the era almost everyone agreed that the most useful, effective, and least toxic diuretic was meralluride given parenterally. Although even today meralluride still retains a high place in any diuretic program, there have appeared some very effective rivals which in certain circumstances have replaced it. These developments have been rapid, involve many complex factors, and unless basic principles are understood may confuse the physician. It is therefore desirable to consider the diuretic agents and establish an acceptable plan for their use. Although there may be some minor disagreement, for the most part, the most effective, widely used, and least toxic diuretics are: meralluride (Mercuhydrin), mercaptomerin (Thiomerin), chlormerodrin (Neohydrin) of the mercurial group, aminometradine (Mictine) and aminoisometradine (Rolicton) of the pyrimidines of the aminouracil type, and acetazolamide (Diamox), chlorothiazide (Diuril), hydrochlorothiazide (Hydrodiuril, Esidrix, Oretic), flumethiazide (Ademol), hydroflumethiazide (Saluron), and benzydroflumethiazide (Naturetin) of the sulfonamide group.

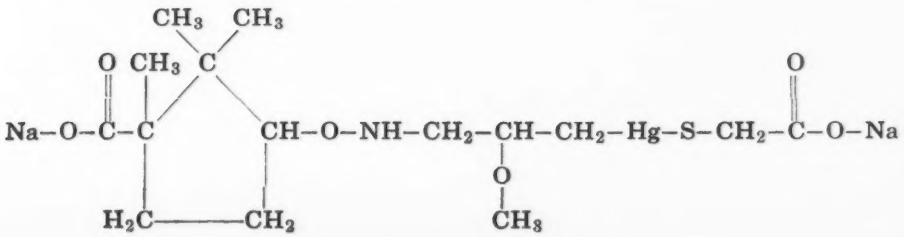
Although the sym-triazines have yielded several active diuretic compounds, only one of the group, chloranzanil (Daquin), has shown sufficient merit and relative lack of toxicity to be used clinically. It occupies a very limited place in therapy at present.

Since the mercurials were the first highly effective diuretics and are still frequently the most desirable in severe, acutely edematous states, it is proper to consider them first. Furthermore, they serve as a standard against which any new diuretic should be compared for over-all effectiveness.

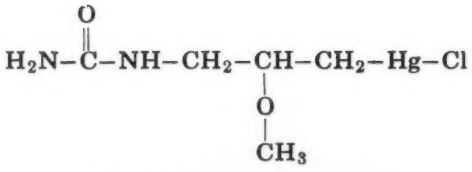
Although there are many mercurials available, clinical usage has concentrated for the most part on meralluride, mercaptomerin, and chlormerodrin. Although there are some newer oral preparations which show merit, their status is as yet experimental.



Meralluride (Mercuhydrin)
(X = theophylline)



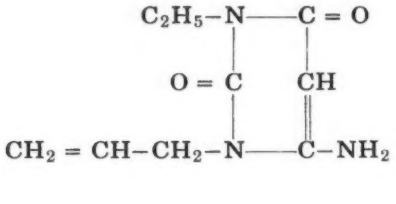
Mercaptomerin (Thiomerin)



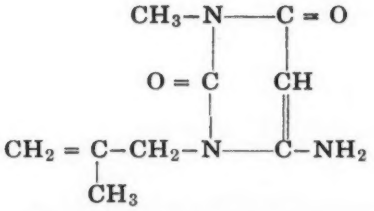
Chlormerodrin (Neohydrin)

The mercurial diuretics have recently been shown by the stop-flow technique of analysis to have their primary site of action in the proximal renal tubules.[1] Their action may involve sulfhydryl groups on renal cell enzymes since they have an affinity for such groups. Their diuretic effect is abolished, if an active agent such as dithiopropanol is given simultaneously. It is, however, possible that mercury reacts with other groups on cell enzymes, such as amino or phenolic structures. The effect on the proximal-tubule cell leads primarily to increased sodium and chloride excretion. Potassium is not significantly altered unless diuresis is vigorous and prolonged. Ammonium, bicarbonate, and phosphate excretion are also not much changed. The effect of mercurials is, therefore, primarily to eliminate water, sodium, and chloride. Tolerance does not develop. Toxic reactions when these agents are used properly are rare. Mercaptomerin exerts less toxic action on the heart and is often selected as the drug of choice if there is severe cardiovascular disease. It, however, is not less toxic to the kidneys or other tissue and has no merit over other mercurials, if there is renal or liver disease. Action with the mercurials begins in 2 hours with maximum effect in approximately 6 hours but continuing for at least 12 to 18 hours. Most of the mercury is eliminated in the first 6 hours and nearly all by 24 hours. The mercurials are consistently effective, potent, disturb homeostasis the least, and are less expensive than most of the newer agents. The chief disadvantage of the mercurials is the necessity for parenteral administration for best results. Chlormerodrin (Neohydrin) is only one-half as effective as meralluride (Mercuhydrin) parenterally. Unfortunately, there is poor absorption of mercury compounds from the gastrointestinal tract (only 8 to 12 per cent) and increasing the oral dose does not enhance the action much, because of this factor, but frequently leads to toxic effects. There is definite need for an oral mercurial which will be readily absorbed from the gastrointestinal tract.

The aminouracils, aminometradine (Mictine) and aminoisometradine (Rolicton), were developed after the mercurials and, although not as effective, they have merit as oral diuretics.



Aminometradine (Mictine)

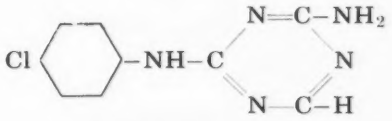


Aminoisometradine (Rolicton)

These pyrimidine diuretics are effective when given orally but are only approximately a little better than two-thirds as effective as parenteral meralluride. Their mechanism of action has not been well established, but the site of action seems to be in the tubules. They tend to produce a hypo-

chloremic alkalosis, but this is mild. Tolerance develops rapidly to aminometradine so that it quickly becomes ineffective on repeated administration and thus for best results should be given intermittently. Aminoisometradine, which has replaced aminometradine, behaves much like aminometradine but much less gastrointestinal distress, nausea, vomiting, and diarrhea have been observed. It is also capable of producing a continuing diuresis, since tolerance is less a problem. Aminoisometradine is given in a 400 to 800 mg. dose four times daily to initiate therapy with subsequent dosage usually reduced to 400 mg. twice daily. There is little disturbance to homeostasis and the drug is for the most part very safe. Occasionally skin rashes occur but the chief difficulty is usually gastrointestinal disturbance. The drug is less effective than parenteral mercurials and some of the oral sulfonamide derivatives and as a consequence is used much less in therapy than it was 3 or 4 years ago.[2]

The next group of agents exhibiting diuretic activity are the sym-triazines. Although the chemistry of the molecule is such as to permit many possibilities, only one so far has exhibited sufficient diuretic activity without prohibitive toxicity to be used clinically. This preparation is chlorazanil (Daquin).

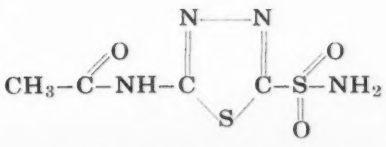


Chlorazanil (Daquin)

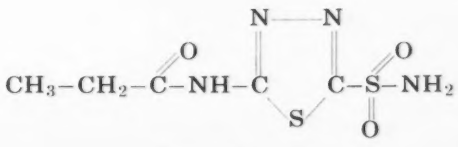
The exact mode of action of this class of agents is also unknown. There is some evidence to suggest a possible primary action to increase water excretion. Sodium and chloride excretion is enhanced, but the potassium elimination is only about half that of sodium and bicarbonate approximately one-fourth that of chloride. Ammonium output is at first suppressed, later increased; phosphate is unaltered. On continuous administration there is a mild sodium loss but no over-all significant change in electrolyte homeostasis.[2]

Unfortunately there may occur an elevation in blood urea nitrogen which probably signifies a toxic effect on the kidney. Because the diuretic effect is only half that of parenteral meralluride, nausea and vomiting which occur fairly frequently, development of tolerance on repeated dosage, and the unexplained elevation of blood urea nitrogen have led to very limited use of chlorazanol. Certainly until more is known about the toxic action on long-term administration of the sym-triazines, they should not be used in the presence of previous renal disease.

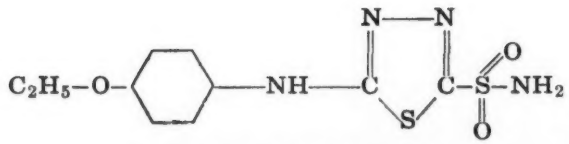
The earliest sulfonamide used to any extent in therapy was the potent carbonic-anhydrase inhibitor, acetazolamide (Diamox). Although others of the series are also potent none of them have as yet gained as widespread use.



Acetazolamide (Diamox)



Butazolamide

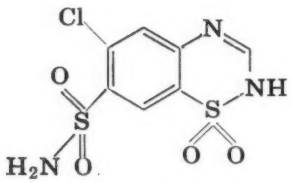


Ethoxzolamide (Cardrase)

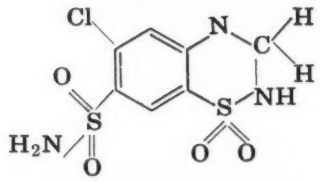
Acetazolamide exerts its action pharmacologically by inhibiting the enzyme carbonic anhydrase and thus interfering with the ability of the renal tubule cells to conserve sodium, potassium, and bicarbonate.[2] Good diuresis accordingly results. Chloride, ammonia, and phosphate excretion remain about the same. Its action begins in approximately 6 hours and may last for 12 hours. Acetazolamide is only about one-fourth as potent as parenteral meralluride. Tolerance rapidly develops to its action; thus for best results it should be given intermittently. A dose every other day or once or twice weekly gives maximum diuretic response.

Continued administration leads to serious potassium depletion and depression of serum potassium and bicarbonate. Its use should be avoided or it should be administered with care in potassium-wasting conditions such as cirrhosis of the liver, nutritional edema, or after steroid therapy. It should be avoided in cases of renal damage with associated acidosis.

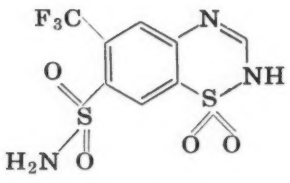
Further search for more potent, less toxic, and more useful sulfonamides led to the discovery of a most interesting series of compounds of which there are at present five in therapeutic use. These agents, all derivatives of benzene-disulfonamide, are the most potent oral diuretics known. They are chemically closely related but slight alteration in structure markedly influences activity.



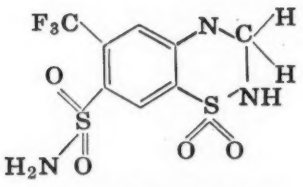
Chlorothiazide
(Diuril)



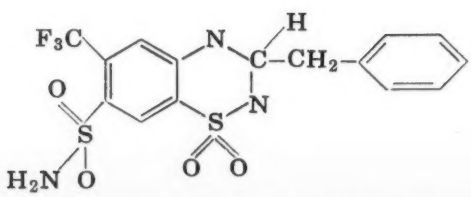
Dihydrochlorothiazide
(Esidrix, Oretic, Hydrodiuril)



Flumethiazide
(Ademol)



Dihydroflumethiazide
(Saluron)



Benzydroflumethiazide (Naturetin)

For example, saturation of the double bond in the thiodiazine ring increases potency by 10 or 15 times and leads to less toxicity. Substitution of a trifluoromethyl group in place of chlorine at the 6-position likewise increases potency 10 times and results in reduced toxicity.

Pharmacologically these agents are all potent diuretics which also have been shown by the stop-flow technique to act on the proximal renal tubule and to produce a diuretic effect very similar to that seen with the mercurials in that sodium and chloride are eliminated about equally while there is much less potassium and only little bicarbonate lost.[4] A mild shift toward hypochloremic, hypokalemic alkalosis is observed. Their potency approximates that of parenteral meralluride and tolerance is not a problem. Even on long-continued use electrolyte homeostasis is not seriously disturbed and toxic effects are limited. These agents all exhibit a hypotensive action which has made them highly useful in the treatment of hypertension. They have been shown to block some of the action of norepinephrine but the exact mode of action, both as diuretics and as hypotensive agents, has as yet not been fully elucidated.[5,6]

Although toxic effects have been limited, there are certain reactions which occur frequently enough to present problems. Skin rashes, generalized or fixed eruptions, and pruritus are not uncommon and resemble those seen with the sulfonamides. Aplastic anemia and agranulocytosis have also occurred. Some patients show hyperuricemia and in a few gouty attacks have apparently been precipitated by these drugs. Abnormal carbohydrate metabolism has also been observed in patients receiving them. Diabetes-like glucose tolerance curves appear, blood sugar levels may be abnormally high, and glycosuria develops in these patients. It is thought that the drugs may unmask an occasional prediabetic patient. Some cases of diabetes are also much more difficult to regulate when chlorothiazide is being administered. Most of these toxic effects are observed when serum potassium levels have become abnormally low on the drug.

Although chlorothiazide was the first of this highly effective group to be used clinically, it is now being rapidly replaced by newer members of the series. Hydrochlorothiazide is approximately 10 times as potent. It is effective, less toxic, and is at present the oral diuretic drug of choice. Hydroflumethiazide and flumethiazide seem at present to be in the same category as hydrochlorothiazide.

Benzydroflumethiazide is the most potent of the series and is the most potent diuretic so far developed. It is highly effective orally. A dose of 2.8 mg. is equivalent to 2.0 c.c. of Mercuhydrin intramuscularly. It causes a significantly increased sodium excretion and there is a decreased loss of bicarbonate. Clinical experience with benzydroflumethiazide is at present limited but early data indicate that it may soon be a diuretic of choice.

These four derivatives of chlorothiazide all exhibit less bicarbonate wastage and seem to disturb electrolyte homeostasis less. Tolerance or refractoriness does not develop.[7,8]

Since there are now highly potent closely related agents, it is worth noting that it is possible to substitute one for another when necessary. We have observed a patient with dermatitis and a fixed drug reaction which develops each time he is given chlorothiazide but when hydrochlorothiazide is given instead the rash disappears and he tolerates the new drug very well,

Table I. *Dosage*

Disease	*Drug*	*Dosage*
Heart disease		
Severe congestive failure	Meralluride	2.0 c.c. intramuscularly daily
	Benzydroflumethiazide	5 to 10 mg. orally daily
Moderate congestive failure	Meralluride	1.0 to 2.0 c.c. intramuscularly daily
	Hydrochlorothiazide	10 to 200 mg. orally daily
	Hydroflumethiazide	50 to 200 mg. orally daily
	Flumethiazide	50 to 300 mg. orally daily
	Benzydroflumethiazide	5 to 20 mg. orally daily
Mild congestive failure and maintenance therapy	Hydrochlorothiazide	25 to 50 mg. orally daily
	Hydroflumethiazide	25 to 50 mg. orally daily
	Benzydroflumethiazide	5 to 10 mg. orally daily
Hepatic disease		
Severe ascites without hypokalemia	Meralluride	2.0 c.c. intramuscularly daily
	Hydrochlorothiazide	25 to 50 mg. orally daily alone or in conjunction with meralluride
	Benzydroflumethiazide	5 to 10 mg. orally daily alone or in conjunction with meralluride
Renal disease	Hydrochlorothiazide	25 to 200 mg. orally daily
	Hydroflumethiazide	25 to 200 mg. orally daily
	Benzydroflumethiazide	5 to 10 mg. orally daily
	Flumethiazide	50 to 300 mg. orally daily
Edema of pregnancy	Same as for renal disease	
Premenstrual edema	Same as for renal disease	
Edema from steroid therapy	Same as for renal disease	

although it varies only by the absence of a double bond in the thiadiazine ring from chlorothiazide. See Table I for dosage and program for diuretic therapy.

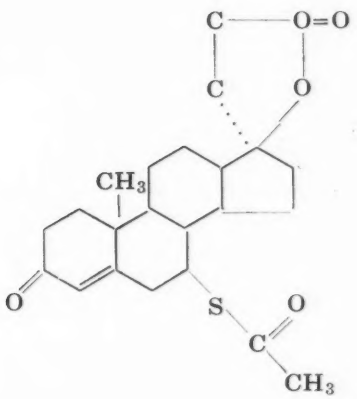
Even though they are not available for prescription use as yet, a few words are in order concerning a new type of diuretic, the aldosterone antagonists. The most promising of this group so far is spironolactone (Aldactone). It exerts an excellent diuretic effect in edematous patients in whom the aldosterone level is high and contributing to the edematous state. Stop-flow analysis has shown that spironolactone acts to block the effect of aldosterone on the distal tubule cells. This leads to an excellent sodium diuresis without loss of potassium. Spironolactone given in conjunction with chlorothiazide enhances the sodium elimination but reduces greatly the potassium loss observed with chlorothiazide alone.

The action is slow of onset and builds to a peak by the third day, persisting for 2 to 3 days after cessation of administration. Studies in numerous patients who have received the drug, some for as long as 6 months, have disclosed as yet very few untoward effects.

Failure to obtain a satisfactory diuretic response when these potent drugs are given properly and in adequate dose may be from a variety of

causes. Certain possibilities should immediately be considered. Has the heart, liver, or kidneys deteriorated so far as to produce irreversible failure?

If not, then an evaluation of the electrolyte status may give the answer. Often hypokalemia has developed which, when relieved by adequate intake of potassium, leads to an excellent diuresis. Perhaps hypochloremic alkalosis and the salt depletion syndrome have developed. The latter is now seen more frequently with the more potent diuretics combined with restricted salt intake. Intravenous sodium chloride in the form of 100 to 200 ml. of a 15 per cent solution given over 3 to 6 hours frequently makes the patient with this syndrome once more responsive to diuretics. Adequate digitalization, correction of anemia, oxygen therapy, and occasionally a day or two of rest from diuretics will result in the return of a good diuretic response.



Spironolactone
(Aldactone)

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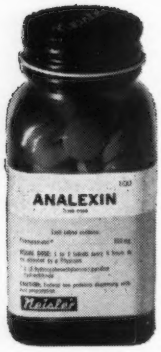

analexin

TEXT ON PACKAGE

Front Panel 100

Analexin Trademark

Each tablet contains:
Phenyramidol*.....200 mg.

Usual dose: 1 or 2 tablets every 4 hours or as directed by a Physician.

*2 (β-hydroxyphenethylamino)-pyridine hydrochloride.

Caution: Federal law prohibits dispensing without prescription.

NEISLER

Right side panel Decatur, Illinois

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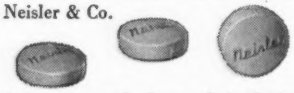
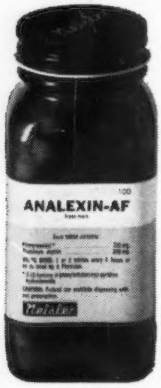
Literature available to Physicians on request

Irwin, Neisler & Co.



Approximate size and shape of Analexin Tablets: Round, compressed, bright yellow tablet imprinted "Neisler".



analexin-AF

TEXT ON PACKAGE

Front Panel 100

Analexin-AF Trademark

Each tablet contains:
Phenyramidol*.....100 mg.
Aluminum aspirin.....300 mg.

Usual dose: 2 tablets every 4 hours or as directed by a Physician.

*2 (β-hydroxyphenethylamino)-pyridine hydrochloride.

Caution: Federal law prohibits dispensing without prescription.

NEISLER

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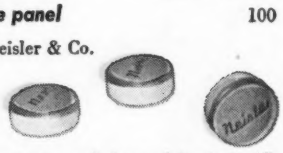
Irwin, Neisler & Co.



Approximate size and shape of Analexin-AF Tablets: Round, compressed, two-layer, two-color (yellow and white) tablet, imprinted "Neisler".

Product Monograph

analexin™
analexin-AF™

For Pain

PHARMACOLOGIC CLASSIFICATION — ANALGOMYLAXANT

Analexin is a new class of drug . . . the first analgomylaxant. It is a new synthetic chemical inherently possessing two different pharmacologic properties: (1) Analexin produces general analgesia by raising the pain threshold, thus decreasing the perception of pain, and (2) it affords muscle relaxation by selectively depressing subcortical brain stem and polysynaptic transmission (interneuronal blockade), abolishing abnormal muscle tone without impairing normal neuromuscular function. Thus in painful states, because pain generates tensions which in turn add to the pain, Analexin can relieve the total pain experience more effectively.

ADVANTAGES

Effective general non-narcotic relief of pain. The analgesic potency of one tablet of Analexin is clinically equivalent to 1 gr. of codeine, but Analexin is non-narcotic and nonhabituating. Tolerance to the drug has not been noted. Muscle relaxant action of Analexin is equal to the most potent oral muscle relaxant available. Thus in painful states the pain and tension which may exacerbate the pain are abolished simultaneously to effectively abate the total pain experience.

INDICATIONS

Analexin is indicated *for the relief of pain* and associated muscle tension in: dysmenorrhea; abdominal distress; genitourinary conditions; tension headaches; gout; dry socket pain, etc.; *for the relief of musculoskeletal spasm and pain* in: low back pain, myalgia, sprains and strains; glass arm; wry neck; osteoarthritis.

Analexin-AF is used *for relief of pain and musculoskeletal tension associated with inflammatory processes and/or fever* as in: arthritis, arthralgia, bursitis, tendinitis, myalgia of strain and tear; and pre- and postoperative toothache.

COMPOSITION

Each **Analexin tablet** contains 200 mg. of phenyramidol hydrochloride 2-(β-hydroxyphenethylamino)-pyridine hydrochloride.

Each **Analexin-AF tablet** contains 100 mg. of phenyramidol and 300 mg. of aluminum aspirin.

HOW SUPPLIED

Analexin and Analexin-AF Tablets are available in bottles of 100 tablets.

DOSAGE

Analexin—1 or 2 tablets every 4 hours. In dysmenorrhea, 2 tablets at onset of pain; then one tablet every 2-4 hours as needed.

Analexin-AF—Two tablets every 4 hours or as required.

PRODUCER

Irwin, Neisler & Co., Decatur, Illinois

INTRODUCED JANUARY, 1960

Current uses of iodides in therapy

What are the present-day indications for the systemic use of iodides? To answer this question an inquiry was sent to some 83,000 physicians* during the spring and fall of 1958, 3,690 of whom replied. The answers tabulated in Fig. 1 indicate that iodides are used extensively for their mucolytic and expectorant action in all common forms of respiratory tract disease.

This impression is confirmed by a glance at the latest literature on the subject, which shows that iodides rank as first choice for liquefying mucus and facilitating expectoration. During recent months, for instance, the merits of iodides were re-emphasized by Sieker[16] for the treatment of asthma, by Bedell and Seebohm[3] and Noehren[11] for pulmonary emphysema, by Segal[15] in bronchitis as a bronchial evacuant, and by Logan[10] in the treatment of children's asthma, confirming earlier recommendations by Glaser[5] and Overall.[12]

While there is no doubt then that iodides are universally used, it comes as a surprise to find hardly any studies published on their mode of action, which has remained rather obscure. This is the more surprising, as there is no lack of publications on the pharmacology and clinical merits of another class of respiratory tract medications, namely, the bronchodilators. Since good expectoration is promoted by both bronchodilation and mucolytic action, the interest in favor of bronchodilators must be considered rather lopsided. It reflects, in part, the rapid development of new agents in the bronchodilator field; but even more, it confirms the old truth that the best things in life are usually taken for granted.

Actually, the "how" and "why" of iodide therapy is still a largely unexplored field, which should reward the investigator who cares to look into it (see Table I.) At least two avenues of research are suggested by observed therapeutic benefits; one is the use of iodides in certain systemic infections, the other concerns the secretion and composition of respiratory tract fluid (RTF).

Systemic infections

For many years iodides held a significant place in the treatment of syphilis. After the introduction of penicillin, Kolmer[8] reassessed their value and rationale. He concluded that iodides are useful against late acquired and congenital syphilis, in which they bring about resolution and absorption of gummatous lesions and exudates.

This lytic action of iodides on granulomatous tissues is not confined to spirochetal lesions; it occurs equally with chronic lesions of different bacterial origin as in pulmonary tuberculosis. It is likely, therefore, that we are dealing here with an effect on the host tissues rather than an attack on the invading pathogens themselves.

Whether systemic iodides possess also a direct spirocheticidal action was studied by Kolmer in experimental syphilis of the rabbit. His observa-

*Survey made through Wampole Laboratories, Stamford, Connecticut.

Table I. *Actions of systemic iodide in infectious and respiratory diseases*

1. *Host modification*	
a. Immediate:	modification and increase of respiratory tract fluid (RTF)
b. Long-term:	lysis of granulomatous tissue
2. *Pathogen modification*	
a.	Increased bacterial sensitivity to penicillin (and to other antibiotics?)
b.	Antimycotic action
3. *Drug action modification*	
Selective concentration in lungs through iodination of:	
	antibiotics (?)
	mucolytic agents (?)
	bronchodilators (?)

tions led to the interesting conclusion[9] that although intravenous or oral sodium iodide alone is not curative, the minimum curative dose of penicillin could be reduced by nearly 50 per cent if sodium iodide was added.

Similar observations were made by Woody and Avery[18] for experimental systemic tuberculosis. They obtained far better results with a combination of iodide and streptomycin than with streptomycin alone.

Of course, one must keep in mind that infectious disease is the combined manifestation of invader action and host reaction, and the observed benefit of iodides may be explained as an action on either of these or on both. In support of there being some direct action on the pathogen is the early report of Jobling and Peterson[7] which stated that iodine will combine with the unsaturated fatty acids from tubercle bacilli, thereby neutralizing their protective ferment-inhibiting properties. It remains likely, however, that the main significance of systemic iodine lies in some action on the host tissues.

Finally, in certain forms of systemic mycoses, a direct antimycotic action appears undeniable. The use of potassium iodide for treatment of pulmonary moniliasis was reviewed by Scott[14] in 1957 and is recommended as the therapy of choice. Scarinci[13] reports cures in 2 cases of chronic primary pulmonary aspergillosis by the combined use of potassium iodide and steroids. Without the additional use of any other agents, this speaks strongly for a specific antimycotic action of systemic iodides.

The secretion and composition of respiratory tract fluid (RTF)

Only a few attempts have been made to explain the mucolytic action of iodides. It is, of course, conceivable that iodides simply stimulate an outpouring of watery secretions in the respiratory tract without necessarily altering the secretion of mucus. The mucus would be diluted under these conditions and thus become less viscous. In favor of such a diluting action is the occasional occurrence of bronchorrhea during iodide therapy. In animal experiments, also, Boyd and associates[4] demonstrated a marked increase of RTF output when iodides or certain organic iodide compounds were given orally. Indeed, there is little doubt concerning the diluting effect of the iodides, either by direct or by reflex stimulation of secretory cells.

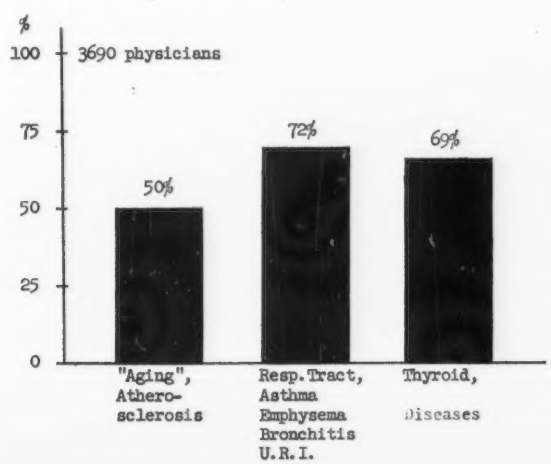
However, the total mucolytic action probably involves more than just that, and there is good reason to postulate that the iodides change the very character of mucus itself at the site of its formation.

Particularly significant in this respect is the selective secretion and concentration of iodides in the lungs. Tuft and Levin,[17] for instance, collected specimens of secretions at different levels of the bronchial tract of their patients by means of the bronchoscope. They found rapid and concentrated excretion of iodine complexes throughout the bronchial tree and through the salivary glands. This selective bronchial excretion occurred well ahead of any significant excretion via the kidneys. This observation was confirmed by other investigators. Boyd and his co-workers,[4] although unable to prove a direct action of iodides on the bronchial glands in rabbits and cats, found that large amounts of iodine complexes are excreted with the RTF. Baker and colleagues[1] performed bronchoscopic studies on 100 patients who received injections of sodium iodide intravenously. Within 15 minutes they found the average iodine value for the bronchial secretions to be 10 times that of the the blood iodide value. Basch and his associates,[2] meanwhile, had demonstrated lowering of pH and decreased viscosity of RTF in response to iodide therapy.

The well-known beneficial action of iodides in asthma, bronchitis, emphysema, and, perhaps, sinusitis is therefore most likely related to selective excretion in the respiratory tract, which in turn leads to increased secretion of thin watery RTF with dilution and possibly chemical alteration of bronchial secretions.

The affinity of iodides for the respiratory tract offers new avenues of research which to date have hardly been explored. An intriguing possibility presents itself in that other therapeutic agents may be selectively concentrated in the lungs if they are linked to the heavy iodine atom. This would obviously be useful in the case of antibiotics, carcinolytic and mucolytic agents. It is worth noting that the iodide of diethylaminoethyl penicillin G (Neo-Penil) is indeed selectively concentrated in the lungs and bronchial secretions. After an injection of 1 million units, average values of 0.6 to

Fig. 1.—Indications for use of systemic iodine by 3,690 physicians.



0.9 unit were detected per milliliter of sputum, in contrast to, at best, 0.06 unit for the iodine-free procaine penicillin.[6] These sputum values are 6 to 10 times more than those found in other tissues. Unfortunately this preparation was too toxic and has no place in therapy in this country at present.

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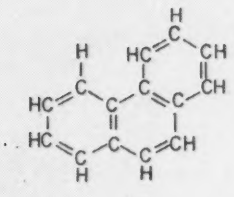
Steroid classification and nomenclature

Recent advances in synthetic chemistry particularly in the steroid field have led to the development of a complicated terminology. Unless one has been closely following developments in the field he is prone to be confused by the symbols, designations, and terms used to identify various chemical entities. Since a thorough knowledge of chemical structure and the importance of changes made in the structure of a chemical compound are essential to proper understanding, it was considered wise to present a brief review of newer substances and their terminology.

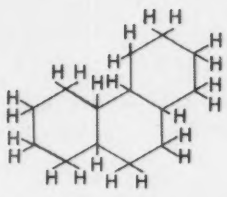
There has also been established a system of a definitive nomenclature for steroids, recommended and described in the *International Union of Pure and Applied Chemistry Nomenclature of Organic Chemistry* (*IUPAC*), Butterworth Scientific Publications, London, 1958. For detailed information reference is made to the excellent texts by Fieser and Fieser,[1] Klyne,[2] and Shoppee,[3] as to the occurrence, nomenclature, and chemistry of the steroids.

The present report is an attempt to provide a simplified grouping of the various hormonal steroid structures and related compounds. The cardiac glycosides and sapogenins are not included. The material is presented in group form so that one may readily recognize the type of activity associated with the various basic structures.

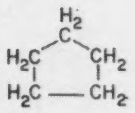
Steroid substances comprise one of the most interesting groups of lipids and constitute a highly specialized division of organic chemistry. These compounds are widely distributed in nature and are of special interest because they possess very specific and potent physiologic effects. In this category are included the sterols, the sex hormones, the adrenocortical hormones, the bile acids, the provitamins D, the steroidal sapogenins, the cardiac aglycones, steroidal alkaloids, and certain carcinogenic hydrocarbons. Historically, cholesterol was the initial compound of this group to be separated and identified. Poulletier prepared it from gall stones in 1769. Its true nature was



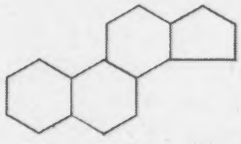
(a)
PHENANTHRENE



(b)
PERHYDROPHENANTHRENE



(c)
CYCLOPENTANE



(d)
CYCLOPENTANOPERHYDRO-
PHENANTHRENE
(SCHEMATIC DRAWING)

Fig. 1.

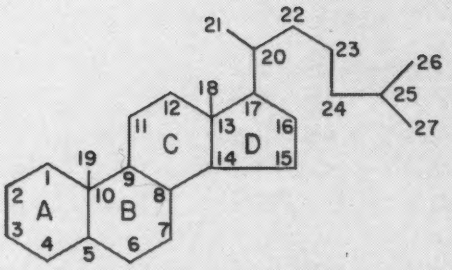
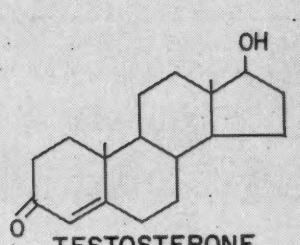
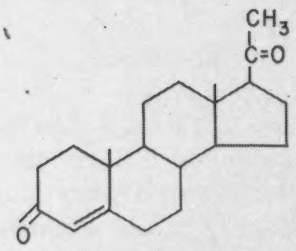


Fig. 2.

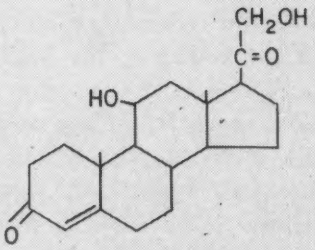


TESTOSTERONE
(17 β-HYDROXY-4-
ANDROSTEN-3-ONE)

Fig. 3.



PROGESTERONE
(4-PREGNENE-3,
20-DIONE)



CORTICOSTERONE
(11 β, 21-DIHYDROXY-
4-PREGNENE-3, 20-
DIONE)

Fig. 4.

first recognized by Chevreul who demonstrated that it was *unsaponifiable* and hence could be differentiated from other waxlike materials. The term "sterol" did not come into general use until 1911 as a term to include all the animal and plant *alcohols* of this series: the sterols are the most widely distributed of the *steroids*. The term "steroid" was suggested by Callow in 1936 and has been widely adopted. Although the present empirical formula of C_{27} $H_{46}O$ for cholesterol was proved in 1888, it was not until the period of the early 1930's that the structural formulas of cholesterol and other sterols were first established. Adolph Butenandt in the 30's established the basic structure for this class of compounds.

The steroid molecule consists of a framework of three six-membered (perhydrophenanthrene) rings and one five-membered (cyclopentane) ring with methyl groups at positions 10 and 13 (Fig. 1).

The method of numbering the carbon atoms and the ring system and side chain is illustrated in Fig. 2.

The compounds in which no side chain is present on carbon atom 17 constitute the "C_{19} series." These include the male sex hormone, testosterone, and its urinary metabolites (Fig. 3).

Steroids with a side chain of two carbon atoms, the "C_{21} series," include the progestational hormone, progesterone, and the hormones of the adrenal cortex (Fig. 4).

In the C_{24} series a branched side chain consisting of 5 carbon atoms is present; this is characteristic of the bile acids. A number of cardiac aglycones contain 23 carbon atoms and several naturally occurring steroids contain 24 carbon atoms (Fig. 5).

Compounds in the C_{27} series contain a side chain of eight carbon atoms, typified by that of cholesterol and provitamin D_3 (Fig. 6).

Steroids containing 28 and 29 carbons are known; most of them are not hormonal and there is little confusion in their nomenclature.

Substituents

The carbon atoms at each of the ring junctions in the steroid nucleus normally carry an additional substituent; methyl groups are present at positions 10 and 13, and hydrogen atoms at positions 5, 8, 9, and 14. The geometry of the carbon to carbon bond joining any two rings permits two spatial arrangements of these substituents, one in which both the substituents are located on the same side of the plane of the ring system (*cis-configuration*) and a second in which the two substituents are on opposite sides of the plane of the ring (*trans-config.*).

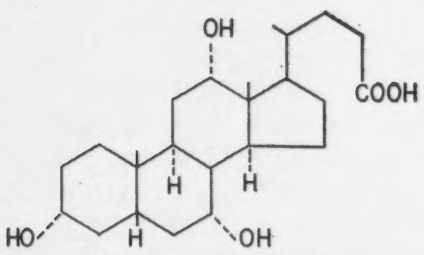
Altogether there are six centers of stereoisomerism in the steroid ring system, indicated by asterisks (Fig. 7).

These could theoretically give rise to 64 stereoisomers calculated on the basis of the number of isomers being equal to 2^n, where n is the number of asymmetric carbons. The number of ring stereoisomers actually encountered in the naturally occurring steroids is considerably less. The steric arrangements at the B/C and C/D ring junctions are identical in all but a very few naturally occurring steroids. All naturally occurring steroids have essentially a flat structure.

The two centers of isomerism at positions 5 and 10 carry a hydrogen atom and a methyl group, respectively, and may give rise to four stereoisomers. These constitute two pairs, one pair with *cis*-configuration and one pair with *trans*-configuration, as shown in Fig. 8. Since the absolute configuration of many steroids is known, the naturally occurring isomers are represented as having the methyl group at position 10 projecting *above* the plane of the ring system, indicated by a solid line; all other stereochemical relationships are referred to this carbon.

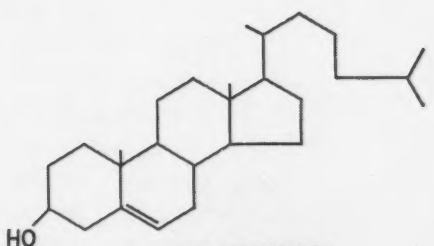
C_{19} or "Androgen series"

The naturally occurring steroids in the C_{19} series may accordingly be regarded as derivatives of the two basic hydrocarbons, *androstane* and *etiocholane*. The lower formulas (*c*) and (*d*) of Fig. 8 represent hypothetical stereoisomeric forms which do not occur in natural products and have not yet been synthesized.



CHOLIC ACID

Fig. 5.



CHOLESTEROL

Fig. 6.

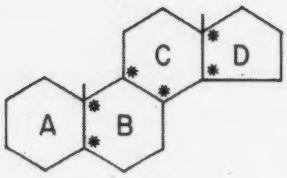


Fig. 7.

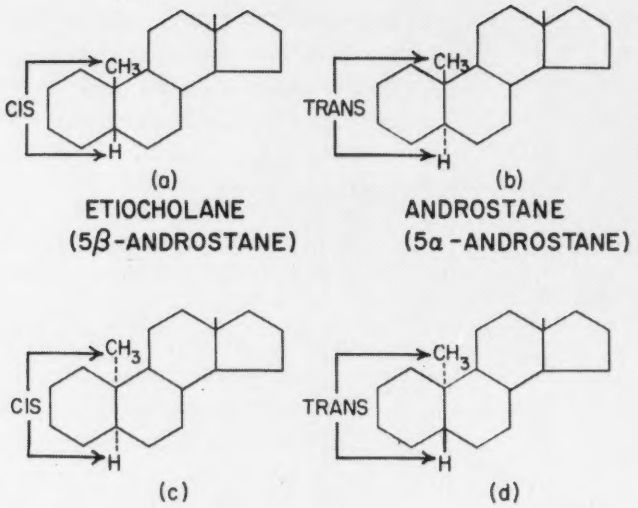


Fig. 8.

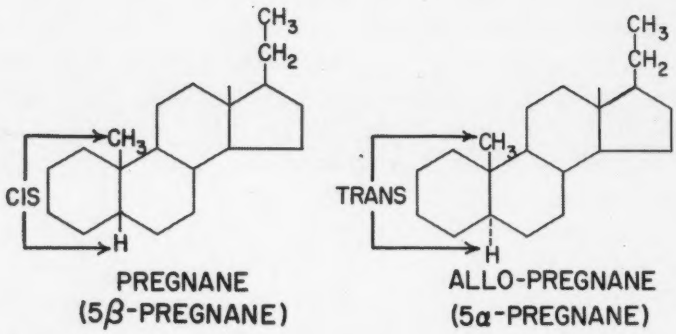


Fig. 9.

C_{21} or "Pregnane series"

The naturally occurring steroids in the C_{21} series may likewise be considered as derivatives of the basic or parent hydrocarbons, pregnane with the *cis*-configuration of the hydrogen on carbon 5, and allopregnane with a *trans*-configuration of the A/B ring junction substituents (Fig. 9). In this group, additional isomerism is introduced by the side chain at carbon 17.

In the methylene (—CH_2—) groups of the ring system, the two hydrogen atoms project one above and one below the plane of the molecule. If one of these hydrogens is replaced by some other group, a new center of asymmetry is introduced. Two structures are possible for the derivative, one in which the group projects below the plane of the ring (indicated by a dotted line).

Many steroids carry a hydroxyl group at position 3. In order to designate the two series of 3-substituted stereoisomers, Fieser introduced the use of the alpha and beta symbols to indicate the stereochemical relationship of the hydroxyl group at carbon atom 3 to the rest of the molecule. The beta (β) configuration was originally defined as that present in the linkage of the hydroxyl group on carbon atom 3 in cholesterol, and the opposite, alpha (α) configuration, as that present in the bile acids and in androsterone. In the beta compounds the hydroxyl group lies on the same side of the ring as the methyl group at 10, and accordingly it is indicated by a solid line. Similarly

substituents in the C and D rings are related to the configuration of the methyl group at carbon *13*. The configuration of the carbon at 13 is the same as that of the carbon at position 10. These 2 angular carbons are numbered C_{18} and C_{19}, respectively (see Fig. 2).

In the C_{21} series, stereoisomerism of the same type may arise at position 17 where the two-carbon-atom chain may project above or below the plane of the ring. The absolute configuration of the side chain in the pregnane and allopregnane compounds had been a subject of controversy, but work of Sorkin and Reichstein (1946) and of Long and Gallagher (1946) indicated that all naturally occurring C_{21} steroids have a beta configuration, with the side chain *cis* to the methyl group at carbon 13 (also carbon 10).

When early studies on adrenal extracts and steroid hormones were being carried out, it became difficult to ascribe all observed effects to one adrenal cortical hormone. Since then a surprisingly large number of crystalline steroids have been isolated from the adrenal, differing qualitatively and quantitatively from one another in their biologic actions. About fifty steroids have been isolated from the adrenal cortex; seven of these have proved to be biologically active; the remainder are either inactive or their biologic activity has not been reported. Except for the androgenic compounds, the physiologically active adrenal steroids contain the Δ^4-3 keto-group and the two-carbon side chain at carbon 17 and are therefore Δ^4—pregnenes (Fig. 10). The majority of the inactive compounds are allopregnanes. Structurally,

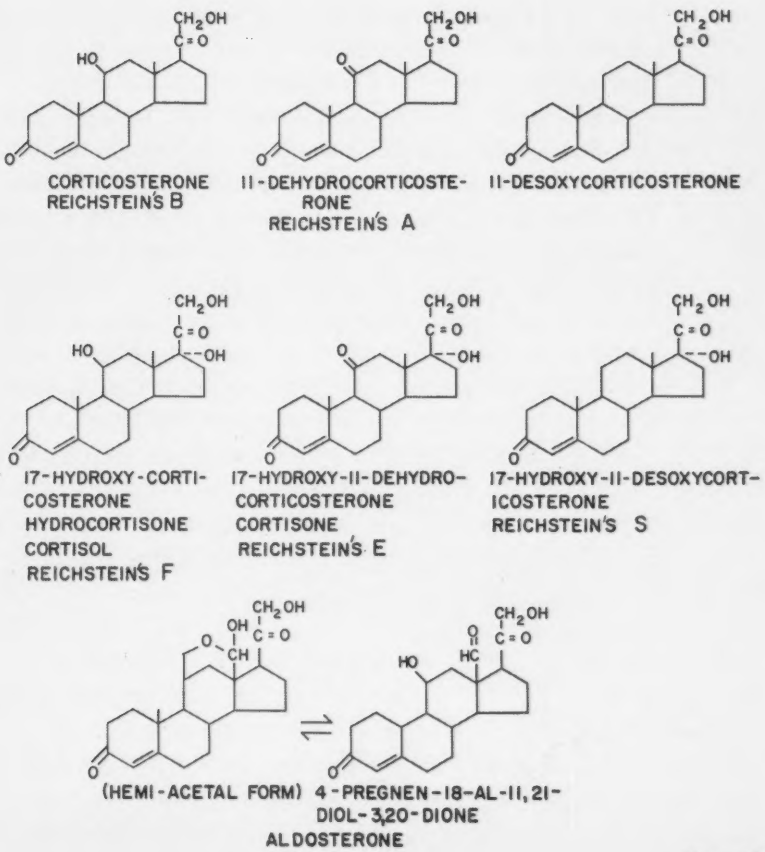


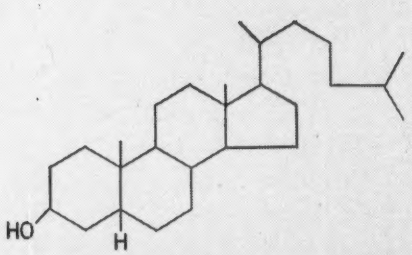
Fig. 10

these active compounds differ from one another only in the number and position of the attached hydroxyl or ketone groups and can be looked upon as being in different stages of oxidation and reduction. It has been fairly well established that these different substances are either derived by differential degradation of one parent substance, and many of the isolated members are intermediate compounds, or the different hormones are built up separately and directly from smaller units. It should be noted that after removal of all the above-mentioned seven crystallizable materials from beef or hog adrenal cortex an amorphous fraction remained in the mother liquor which contained the major part of the original salt-retaining activity.

In 1954, Reichstein in Switzerland isolated a steroid from this active adrenal residue which has physiologic properties similar to those of desoxycorticosterone but is many times more active. From 1,250 pounds of adrenals he and collaborators were able to obtain 22 mg. of crystalline material. Because of its sodium-retaining properties, this compound was referred to as "electrocortin" or the salt hormone and named aldosterone. The structure of this compound was established by the same Swiss group. Since that time several workers in the field have reported the identification of this compound in urine. This makes seven biologically active compounds (which have been isolated) from the adrenals. Besides being referred to by generic or trivial names, the cortical steroids are referred to by letters given to them before their true nature was established.

C_{27} or "Sterol series"

Cholesterol was the first recognized and also is the most important member of the C_{27} steroid group (see Fig. 6). Stereoisomerism is a most important property of cholesterol and related sterols. The most important point of asymmetry of cholesterol itself is at carbon 3, while a second important focal point for such cholesterol derivatives as cholestanol or dihydrocholesterol and coprostanol is established at position 5 (Fig. 11). Carbon 5 is not an asymmetric one in cholesterol, because of the double bond between carbons 5 and 6. However, on reduction of cholesterol, asymmetry develops at C_5, and two important classes of naturally occurring steroids can be prepared, depending upon the configuration around this carbon. The hydroxyl group on carbon 3 assumes a position similar to that in cholesterol in dihydrocholesterol, stigmasterol, ergosterol, sitosterol, and fucosterol. Only the compounds which possess this configuration for the hydroxyl group (called beta) are precipitable by digitonin, a saponin which is a combination of a steroid with sugars. Digitonin forms an ether-insoluble complex with *3-beta*-hydroxy-sterols.



COPROSTEROL (COPROSTANOL)

Fig. 11.

The two classes of stereoisomers in which a difference in configuration exists on the C_5 atom are called the *normal* and the *allo-series*. Coprosterol, a natural saturated sterol formed from cholesterol, which is present in feces, belongs to the *normal series*; dihydrocholesterol, which is prepared by reduction of cholesterol, is a member of the *allo*-series.

The configuration characteristic of the hydrocarbon skeleton of cholestanol (dihydrocholesterol) is termed the *allo* configuration. Steroids of both the normal (or *cis*) and of the allo (or *trans*) configurations have been found in nature. The differences are not concerned with the arrangements of the atoms and groups around carbon 3, since they are still maintained in the compounds formed when coprosterol and dihydrocholesterol are reduced to their corresponding hydrocarbons, namely, *coprostane* and *cholestane*.

The methyl group at carbon atom 10 is also used as the reference point in defining this group of compounds. Compounds in which the hydrogen and methyl groups are in *cis* relationship are considered to be the normal series, while those in which a *trans* relationship exists between these groups are referred to as the *allo* series.

Hydroxyl groups are present in many steroid hormones and their isolated metabolites. In addition to the 3 position, hydroxyl groups are frequently observed also at positions 17 and 21. Many of the steroids derived from the adrenal cortex also carry a hydroxyl group at position 11 as well as a hydroxyl at 21 as part of the characteristic *ketol* (combination of *ket*one at C-20 and alcoh*ol*) side chain. In the bile acids hydroxyl groups usually occur at carbons 3, 7, and 12.

The ketone group occurs in many steroids usually at the same positions as the hydroxyl group, i.e., 3, 7, 11, 12, 17, and 20. There is considerable evidence to show that, in the course of metabolism, oxidation and reduction involving the hydroxy groups and ketone groups at various positions in the molecule may take place. In the naturally occurring steroids the carboxylic acid group is present in the bile acid side chain.

An additional factor which contributes to the complexity of the naturally occurring steroids is the frequent presence of *ethylenic unsaturation* in the molecule. Isolated double bonds can occur at a variety of positions. They are found frequently at the 5 position, as in cholesterol and in dehydroisoandrosterone, and have been introduced at the Δ^1, Δ^2, Δ^4, $\Delta^{9:11}$, Δ^{11}, and Δ^{16} positions, and in the side chain.

In the literature, the symbol Δ has been used to indicate the position of a double bond in the molecule. Only one numerical superscript is shown if the opposite end of the double bond is attached to the carbon atom carrying the consecutive higher number, otherwise both ends of the bond are indicated, the numbers being separated by a colon or the last figure enclosed in brackets. In the case of the diene, a comma is used to separate the numbers locating each of the two bonds. Recently it has become customary to eliminate the symbol Δ and use a common root name immediately preceded by the number which indicates the position of unsaturation.

Conjugated ethylenic double bonds are found also in 7-dehydrocholesterol, the precursor of vitamin D_3, and in the urinary metabolite, 3,5-androstadien-17-one. Conjugation of an ethylenic double bond with a ketone group very frequently occurs, particularly in the Δ^4-3-ketone arrangement, which is found in progesterone, testosterone, and the active steroids of the adrenal cortex.

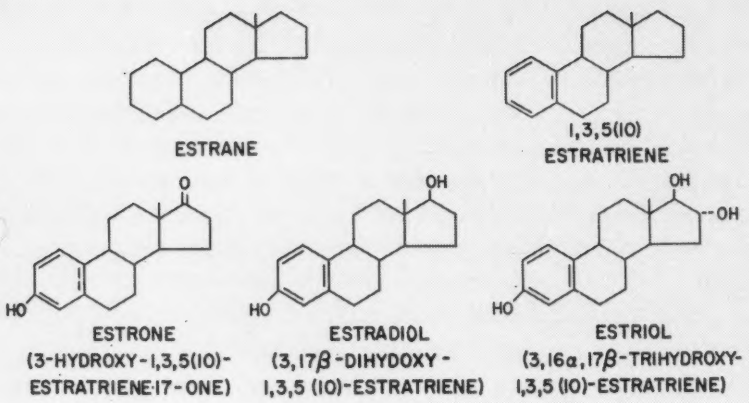


Fig. 12.

Estrogens

The estrogens, which are formed of *monomethylated* steroid nuclei, are derived from the parent compound, estrane. Since they are aromatic in ring A, the common estrogens are named systematically as derivatives of 1, 3, 5 (10)-estratriene (Fig. 12).

The presence of an aromatic ring A is characteristic of the estrogens. In the aromatization of ring A, carbon atom 19 is eliminated, therefore this group of steroids contains 18 carbon atoms. They may be looked upon as members of the C_{19} steroids in which one of the carbons has been eliminated. The aromatization of ring A confers phenolic properties on the hydroxyl group at 3. Frequently a compound in which a carbon has been eliminated, either from the positions at carbon 10 (elimination of carbon 19), at carbon 13 (elimination of carbon 18), or from one of the rings is referred to as a "nor" compound. "Nor" was derived from the German terminology used in naming nitrogen-containing alkaloids, in which the elimination of a radical from the nitrogen (N) was referred to as "*N ohne radical.*"

Current recommendations for nomenclature in the steroid field prefer to eliminate the use of "etio-" and "allo-" in designating structural differences involving the configuration of the hydrogen attached to carbon atom 5—i.e., using "5β-andro-" and "5α-pregn-" for "etio-" and "allopregn-". Although it is desirable that investigators take cognizance of these suggestions, one would have difficulty in finding his way about in the vast literature of the steroids by using only current nomenclature. Among the reasons for confusion have been the periodic changes suggested and the resistance of authors to breaking away from the use of accepted trivial names. The current trends in steroid synthetic chemistry are to alter substituents and make slight modifications in the basic molecule. These minor changes in the molecule are responsible for unusual biologic alterations of the parent compound, resulting, as an example, in new C_{19} steroids (traditionally referred to as androgenic steroids) which have progestational or corticoid biologic properties.

Although the actions of these hormones differ widely, their basic chemical structure is quite similar in that they possess a common nucleus. Minor

differences in their molecular structure, such as the number of double bonds, the presence of side chains (and the addition of simple constituents to the rings or side chain), account for striking differences in biologic activity of the steroid hormones.

Glossary

Prefix	*Meaning*
allo	5α orientation of hydrogen
anhydro	loss of water as H and OH from adjacent carbon atoms
dehydro	loss of hydrogen
deoxo or desoxo	loss of O, usually from an OH
deoxy or desoxy	loss of an OH group, replaced by hydrogen
dihydro	addition of 2 atoms of hydrogen
epi	inversion of a substituent; example—3β-hydroxy "epimerized" to 3α-hydroxy
homo	addition of CH_2 as in a homologous series; usually used in describing an expansion of a ring
iso	inversion of a substituent group; usage now being replaced by epi
nor	loss of carbon; as in contraction of a ring or shortening of a side chain by CH_2; as in loss of CH_3 as angular methyl group

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The antihistamines

The most effective drugs for the relief of acute allergic rhinitis have been developed from the antihistamine class of agents. These compounds antagonize many of the actions of histamine. When given in adequate amounts, they will prevent histaminic shock, bronchospasm, and whealing on the skin. They do not completely block the effect of histamine on all organ receptors, since histamine stimulation of salivation and gastric secretion are not inhibited. They also have antianaphylactic properties in large doses and are antipruritic and analgesic. Some have bowel or bladder smooth muscle antispasmodic action; some produce sedation and others central nervous system stimulation. Unquestionably, only a portion of these effects can be ascribed to their antihistaminic action, but the term antihistamine is generally suitable and has received wide acceptance in medicine. It is thought that the mechanism of histamine antagonism is one of competitive inhibition with these agents attaching to or blocking receptor sites.[2,3]

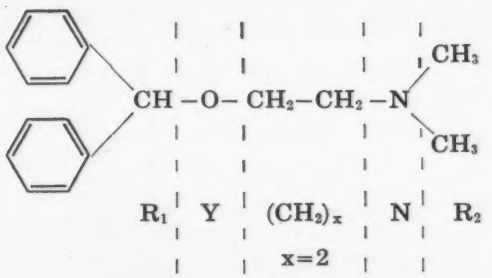
The first widely used compounds of this class were diphenhydramine (Benadryl) and tripelennamine (Pyribenzamine), which appeared in 1946 and 1947, respectively. Since then, many drugs of this class have been introduced. There are now at least twenty-four which are used in varying degrees in therapy. This plethora of agents makes selection of the more desirable difficult and presents a problem to the clinician. There are pronounced differences among the antihistamines in potency, length of action, undesirable effects, cost, and toxicity. Knowledge of these factors is essential for proper drug selection and will guide the physician in his choice.

Certain fundamental principles related to chemical structure and pharmacologic action also have been observed. By making use of these, the physician can, with certain limitations, assess intelligently the probable type of action to be expected. Antihistamine drugs, in general, conform to a rather simple chemical pattern. This pattern may be expressed schematically as follows:

$$R_1-Y-(CH_2)_x-N-R_2$$

R_1 may represent a single, two similar, or two dissimilar groups. Y is a linking radical which may be carbon, nitrogen, or oxygen. In the radical, $(CH_2)_x$, x may be one or two. N united with (CH_2) usually as a substituted ethylamine is essential. R_2 may be a ring complex or two similar or two dissimilar groups.

The structure of diphenhydramine, the first widely used of the series, illustrates the above principles:

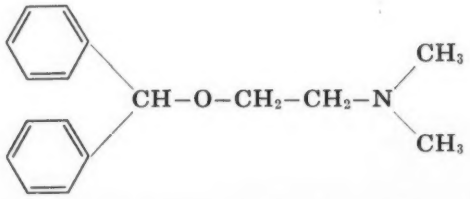


As newer antihistamines with many variations in chemical structure, but all conforming rather closely to the above pattern, appeared and were used clinically, certain principles became evident.[1] (1) Substitution of a chlorine or bromine on the phenyl group in R_1 enhances potency. (2) In compounds with optical stereoisomerism, the dextrorotatory fraction contains most of the action. (3) Compounds with oxygen as the linking radical are likely to have more sedative action than those with carbon or nitrogen. (4) The $(CH_2)_x$ group may be absent—one or two, but never three. Most compounds in clinical use have two. (5) The most common N-R_2 group is dimethylamine. (6) A minimum molecular weight of 150 is necessary for active compounds.

Although there are available over two dozen antihistamines, clinical usage has for the most part concentrated on five or six. There are at least eight principles which should be kept in mind when selecting an antihistamine. (1) It should be potent. (2) Is a sedative or stimulating compound desirable? (3) What length of action is best or desired? (4) Are there undesirable effects on the eyes (glaucoma), bowel (constipation), or bladder (urinary retention)? It may be necessary to use weaker antihistamines in some patients in order to avoid undesirable effects. (5) Have serious reactions such as blood dyscrasia, mental disorientation, or allergic reaction been observed, and if so, with what frequency? (6) Is tolerance or habituation a problem? (7) Does the drug give the desired response in the patient needing relief? (8) Cost must always be considered. The cheaper drug may, depending on the patient's response, prove to be the most effective while the more expensive drug may not only prove to be not much more effective or of such small degree of increased effectiveness as to not be worth the added cost.

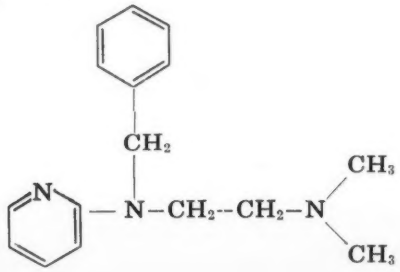
Diphenhydramine and tripelennamine are widely and justifiably used not only because they were the first drugs of the series but also because they are highly effective, very useful antihistamines.

Diphenhydramine



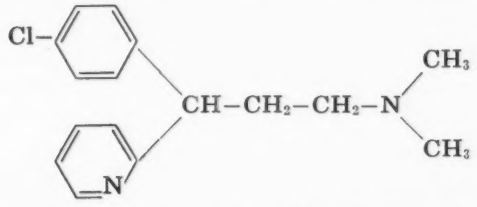
in particular exerts a sedating, centrally depressing effect which is most useful, and many patients find this desirable. These actions frequently make diphenhydramine very useful in controlling vasomotor rhinitis, hay fever, and some pruritic conditions, especially when the itching is at night. It is also useful in reducing vertigo, nausea, vomiting, and some of the unpleasant rigidity of Parkinson's disease.

Tripelennamine



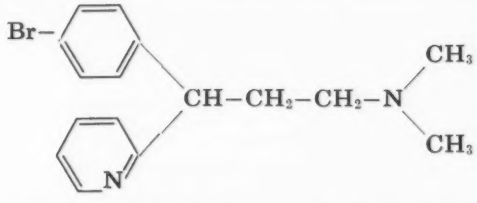
is considered by many to be as effective as diphenhydramine as an antihistamine for hay fever and vasomotor rhinitis, but without as much sedating action; in some patients it may actually give a stimulating effect. It has also been found to be somewhat less useful in vertigo, nausea, and vomiting and as an antiparkinsonism agent than diphenhydramine. It is, however, one of the best antihistamines and has wide use in therapy.

One of the more recently introduced, highly effective, and widely used drugs of the series is chlorpheniramine (Chlor-trimeton)



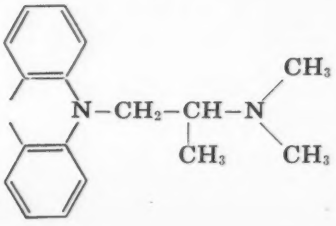
made by substitution of chlorine on the phenyl ring of pheniramine. This is one of the more potent agents and is for the most part free from serious toxic effects. It creates very little sedation or other untoward effects and at present is considered by many to be the antihistamine of choice. Recently, resolution of it into its two stereoisomers has shown that the dextroisomer contains almost all of the activity of the racemic compound. The dextroisomer has recently been introduced as dextrochlorpheniramine (Polaramine).

The bromine-substituted derivative of pheniramine, brompheniramine (Dimetane)

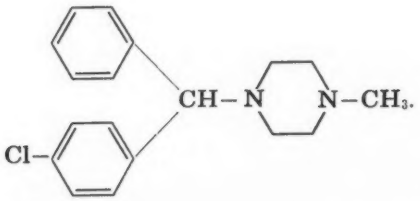


and its dextrorotatory isomer, dextrobrompheniramine (Disomer), have also been recently made available. These preparations are effective and there is merit in that the dextrorotatory preparations are effective in low doses. This reduces the amount of body exposure to foreign molecules and will perhaps reduce drug toxicity. Clinical work so far indicates that they have a low incidence of side effects. They are, however, expensive and will do no more than the parent racemic preparation for most patients. Furthermore, in the bromine-containing compounds, some feel there is always the possibility of bromine sensitivity with skin eruption similar to that seen with iodide-containing preparations. No such reactions have as yet been reported, and with the tiny dose given such a reaction would undoubtedly be rare.

There is certainly need for long-acting antihistamines and there are at least two excellent ones available. These are the phenothiazines, promethiazine (Phenergan)



and chlorcyclizine (Di-Paralene, Perazil)



These agents may be given once every 8 to 12 hours because of their prolonged action. Promethiazine exerts considerable sedating effect and, being a phenothiazine, must be observed during use for the toxic reactions seen in that class of agents, although as yet few serious reactions have been reported. It exerts an antiemetic effect, has a tranquilizing action, and is a useful adjunct when its potentiating action on analgesic and sedative drugs is desired. The sedation may be so severe as to create drowsiness and dizziness and lead to sleep during the day. Therefore, ambulatory patients must be cautioned that skilled activities may be impaired.

Absorption and elimination

The antihistamines, as a class, are readily absorbed by mouth, with action beginning in 15 minutes, reaching a peak in 1 hour, but persisting in an effective degree for 3 to 6 hours. Promethiazine and chlorcyclizine, however, may give sustained effective action for as long as 8 to 12 hours.

In the body, they are metabolized or converted into inactive substances, since very little active material is excreted by the kidneys. The liver, certainly in the case of promethiazine and probably for others of the series as well, is the chief site of inactivation.

Toxicity

Although the antihistamines, as a class, exhibit a low degree of toxicity, there are certain untoward effects common to them that must be borne in mind during their use.[4] Skin allergy to topical application is of such a high degree that it is best to avoid their use in this manner. They frequently cause sedation which, in certain patients, is of sufficient severity to present a hazard to those in positions or activities requiring alertness or close attention. Since the degree of sedation varies with the individual, it is absolutely essential that the physician carefully instruct and see that each patient understands this and ascertains his reaction to the drug before placing himself in situations where serious harm to himself or others might occur.

Dryness of the mucous membranes of the nose, mouth, and throat can be pronounced. Although this effect is usually of little consequence, it can lead to a bad taste, decreased sense of taste and smell, and, in some patients, cough, throat irritation, nausea, and loss of appetite. Occasionally a patient may complain of a lump in the throat and develop a syndrome similar to globus hystericus while on these drugs as a result of the throat dryness. Tachycardia, premature systoles, and runs of premature systoles may occur. Nausea, vomiting, pyrosis, and diarrhea or constipation have also been seen, although these are uncommon.

In large doses and occasionally also when given in ordinary doses, they

are capable of producing palpitation, nervousness, agitation, insomnia, excitement, delirium, disorientation, confusion, hyperpyrexia, and convulsions. In the very young, the elderly, and the patient with impaired liver function, these reactions are most commonly observed.

Agranulocytosis, leukopenia, and other evidence of bone marrow depression have been reported, but these reactions are very rare and were for the most part seen in patients receiving the less commonly used agents or those drugs given in a dose of 25 to 50 mg. three, four, or more times a day.

As with many of the antiparkinsonism drugs, some of which are closely related to the antihistamines, the atropine-like effect, although feeble, may on long, continued use be detrimental to the patient with glaucoma.

Therapeutic uses

In therapeutic uses, probably the most effective results are obtained when the antihistamines are employed in the treatment of urticaria and angioneurotic edema. Approximately two-thirds of the patients with acute or chronic urticaria and those with angioneurotic edema will secure temporary effective relief.

Allergic rhinitis of the episodic seasonal type responds surprisingly well and frequently the antihistamines are so effective as to afford nearly complete comfort from this very disagreeable allergic state. Chronic vasomotor rhinitis, however, is less effectively managed and at least half of the patients get little or no relief. In bronchial asthma, their use has been disappointing, in that less than one-fourth secure any relief. Furthermore, the drying action produced on mucous membranes is frequently undesirable and may make the asthma worse.

Pruritus of the vagina or rectum and, less often, neurodermatitis are alleviated to a modest degree with the more potent, sedation-producing antihistamines, such as diphenhydramine, given at bedtime. Serum sickness reactions are markedly reduced by adequate and continued antihistamine therapy.

Prophylactic use of antihistamines such as chlorpheniramine, diphenhydramine, and tripelennamine, when added to infused blood, has been successful in reducing the incidence of transfusion reactions. Certainly, intravenous preparations of these should be available for emergency use not only in blood transfusion reactions but also in severe reactions from other drugs and solutions whether given intravenously or by other routes.

There are many other less common uses for the antihistamines. Tripelennamine is employed as a safe, useful topical anesthetic in urologic conditions. Diphenhydramine in particular and others to a lesser extent are useful in the treatment of Parkinson's disease. Promethiazine has wide use as a tranquilizer, antiemetic, and potentiating agent in anesthesia.

Although antihistamines particularly in conjunction with analgesics have widespread use in the treatment of the common cold, it is questionable if this is wise therapy. They unquestionably in many patients do dry up the profuse watery nasal discharge and give temporary amelioration of the symptoms. In a substantial number of individuals, however, the mucous membrane drying effect leads to crusting, congestion, increased irritation, and not infrequently to a more severe and prolonged course, with sinusitis as a complication occurring all too often.

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Sedative hypnotics

The selection of a suitable sedative hypnotic type of drug poses many problems. Many questions arise as to when and which to use and what may be accomplished by this therapy. The needs vary not only with the patient but with individual fluctuations of the patient's medical status. This inevitably leads to difficulties in the initial selection of a sedative and, furthermore, frequently calls for rapid changes in the type of medication being given. It is imperative that, for best relief, the drug be fitted to the individual patient and his particular needs at the particular time in his medical history. The needs of the patient, his response and the varying modes of action, degrees of effectiveness, metabolism, toxicity, and finally the cost must all be considered when selecting the agent to be used. It is becoming increasingly important that the proper drug be fitted to the patient's needs and used only for the period required.

There is altogether too much use being made of this class of drugs, both by physicians and by the lay public. If used wisely, they certainly are a helpful means of controlling undesirable central nervous system tension states. Used unwisely, they can prove to be a definite drawback and may be harmful to the individual taking them. The decision to use these drugs must come only after careful consideration of the patient's needs. They are not curative in themselves but may be used along with other more definite therapy as an aid to the patient's recovery. Frequently, sedation is prescribed when a more careful evaluation would show that little could be accomplished from such medication. Unfortunately, some patients use the sedative hypnotics as a crutch to help them in the struggle against the everyday pressures of living. The increased consumption of these agents and the larger number available with new ones appearing every few months have certainly made this class of compounds one of the more important in therapy. They have headed the list of prescription drugs for several years and there does not seem to be any noticeable reduction in their use since the advent of the tranquilizing agents.

Unquestionably, many of the sedative hypnotic drugs are effective and certainly useful in the treatment of patients when properly selected and adjusted to the patient's needs.

There are many situations where the sedative hypnotics are useful in therapy. In general these needs fall into eight categories. A brief survey of them may be of interest.

1. The foremost use is undoubtedly in the sudden, limited, stressful situation where a great emotional strain has suddenly been thrown upon the individual and his normal ability to cope has been overcome for a limited period. These patients suffer severe mental and emotional strain, are unable to sleep, and suffer from fatigue and physical exhaustion. The use of a sedative often proves most helpful.

2. Chronic tension states created by disease or sociologic factors, such as pressure at work, unfavorable working conditions, home or family situa-

tions, may require sedatives while more definitive therapy is being carried out. In these patients, the greatest care must be exercised in the use of sedatives to avoid doing harm since, in this group, dependence and ultimately addiction can develop if drugs are used unwisely.

3. In hypertension, sedation may be desirable until the patient has learned to lead a more relaxed existence and his hypertension has been controlled by other drugs.

4. They are useful in potentiating the analgesic drugs and in helping control the severe central nervous system effects of pain.

5. Their value in controlling convulsions from various causes is well established.

6. They have widespread use in surgery and obstetrics as adjuncts in anesthesia.

7. Certain of the agents are useful in psychiatry for their role in narcoanalysis.

8. They also have a role in counteracting the effects of other central nervous system–stimulating agents when such agents are necessary in the management of the patient.

Unquestionably, there are other uses, but most of the sedatives are employed in the above situations. It is in the stressful situations in particular that the most opportunity for error in use and abuse of these drugs may arise. In the remaining situations, their use is more limited and better controlled, and the need quickly disappears.

Central nervous system overactivity resulting from disease, stress, or drugs is usually manifested by agitation, insomnia, and physical fatigue. Usually, insomnia is the most distressful and troublesome to the patient, and it is most frequently this symptom that causes him to seek help. In the treatment of insomnia, it is necessary to give some thought to the type and pattern before one selects a sedative hypnotic agent. There are several patterns of insomnia that are fairly well recognized. In general, most patients with insomnia fall into one of four large groups.

1. The first group consists mainly of those individuals who are vigorous, active, and under considerable tension, who seem to continue to build up more and more central nervous system stress as the day goes along so that by evening, when they retire, they are wound up tight and cannot seem to let go of all the day's activities. As a consequence, they toss and turn for an hour or two until they finally sink into exhausted deep sleep for the rest of the night. In this group are also found the patients suffering from sudden severe stressful situations. Obviously, patients in this category require a sedative which will act promptly and be readily dissipated within 2 or 3 hours.

2. The second group consists of those individuals who are the exact opposite of the first. These frequently are older patients, those with arteriosclerosis, patients with a weakened physical condition, and many of those suffering from chronic stress. They become more and more exhausted as the day wears on, and as evening comes, they are exhausted and have great difficulty in keeping awake. They frequently fall asleep in their chairs after the evening meal or are so exhausted they can hardly wait to get to bed. Once in bed, they promptly fall into a deep sleep and usually do well for the

following 4 to 6 hours. Unfortunately, however, they awaken very early in the morning, often at 4 or 5 o'clock. They have a distinct tendency to turn night into day. Frequently, they get up and prowl around the house and do minor tasks in the early hours of the morning. These patients need a sedative which may be slow in taking effect but sufficiently long acting to cover the early morning hours.

3. The third group consists of individuals who frequently have some physical condition or process as the basis of their insomnia. They are often exhausted by bedtime and fall asleep promptly, only to be awakened every 2 or 3 hours by their physical condition. These persons have an intermittent type of insomnia which, although seen in normal persons, is often found in patients who have (1) a low grade fever leading to uncomfortable night sweats, (2) inflammation of a joint which gives no pain when splinted by muscle spasm but which becomes quite painful when the muscles relax in sleep, (3) arteriosclerotic disease causing muscle irritability with cramps, pain, or other uncomfortable sensations in the legs, (4) myalgia or neuralgia which grows worse as the patient relaxes muscles and painful areas are triggered into action, or (5) menopausal change with the associated vasomotor phenomena of estrogen deficiency. Many female patients awake with starts, hot flashes, or sweating episodes several times a night. Patients in this group not only need a different type of sedative but frequently need analgesics, muscle-relaxing drugs, or estrogens.

4. The fourth group consists of another category of individuals with insomnia who very honestly believe that they are getting no sleep or a very limited amount of sleep during the entire night. They are able to hear the clock strike all hours of the night and anything that happens in or outside the house comes to their attention; as a consequence, they feel that they have not actually had any sleep. Unquestionably, many of these patients do get sleep but are awake for long periods. Probably what most frequently happens to these individuals is that they go in and out of sleep many times a night, sleeping a few minutes, then being awake or partially awake for a few. Since they are only able to recall the things that occur during the conscious periods, it seems to them that they have been awake all night. In reality, most of these people sleep 50 to 75 per cent of a night. Of course, it is not a refreshing sleep. In this group are the anxious, tense individuals, many with some psychologic abnormality. Most of them are in good physical health. Agents that deepen sleep and prevent the many episodes of wakefulness are a welcome relief. In order for an agent to be effective in this condition, it also must be one with sufficient length of action to exert an effect throughout most of the night.

After the physician has carefully evaluated the situation and decided that his patient is in need of sedation, he must select the best sedative hypnotic agent for the particular needs of his patient. There is no lack of agents; some twenty-six are at present in active use. It is difficult for the physician to know just which is the better one to use. In general, sedative hypnotic agents can be divided into four categories: (1) those with immediate and very short duration of activity, (2) those with rapid onset and short activity, but with somewhat longer duration than in group 1, (3) those with less rapid onset and moderate duration of activity, and (4) those with slower onset and a long duration of action. (See Table I.)

Table I

Drug	*Sedative dose*	*Hypnotic dose*	*Usual onset of action (min.)*	*Duration*	*Metabolism*	*Usually best therapeutic uses*
Amobarbital (Amytal)	20-40 mg.	0.1-0.3 Gm.	20-30	Intermediate	Liver	Groups* 1, 3, 4
Aprobarbital (Alurate)		65-130 mg.		Intermediate	Liver and kidneys	Groups 1, 3, 4
Barbital		0.3 Gm.	60	Long	Kidneys	Groups 2, 3, 4
Butabarbital (Butisol)	15-30 mg.	0.1-0.2 Gm.	30-60	Intermediate	Liver and kidneys	Groups 2, 3, 4
Butethal (Neonal)		0.1-0.2 Gm.	30-60	Intermediate	Liver	Groups 2, 3, 4
Chloral hydrate	250 mg.	0.5-2.0 Gm.	10-20	Intermediate	Liver and kidneys	Groups 1, 2, 3, 4
Cyclobarbital (Phanodorn)		0.1-0.3 Gm.	15-30	Short	Liver and kidneys	Groups 1, 3
Cyclopentylallyl-barbituric acid (Cyclopal)	50-100 mg.	0.1-0.4 Gm.	15-30	Short	Liver and kidneys	Groups 1, 3
Diallylbarbituric acid (Dial)	30 mg.	0.1-0.3 Gm.	15-30	Intermediate	Liver and kidneys	Groups 1, 3, 4
Ethchlorvynol (Placidyl)	100-200 mg.	0.5-1.0 Gm.	30-60	Intermediate	Liver	Groups 2, 3, 4
Ethinamate (Valmid)		0.5-1.0 Gm.	15-20	Short		Groups 1, 2, 3
Glutethimide (Doriden)	250 mg.	0.5-1.0 Gm.	20-50	Intermediate		Groups 2, 3, 4
Hexobarbital (Evipal)		0.26-0.52 Gm.	10-20	Short	Liver	Groups 1, 3
Heptabarbital (Medomin)	50-100 mg.	0.2-0.4 Gm.	20-40	Short	Liver	Groups 3, 4
Isobutylallyl-barbituric acid (Sandoptal)		0.2-0.6 Gm.	20-30	Intermediate	Liver	Groups 1, 3, 4
Mephobarbital (Mebaral)	30-100 mg.		30-60	Long	Liver	Daytime sedative and anticonvulsant
Methyprylon (Noludar)	50-100 mg.	0.2-0.4 Gm.	15-30	Intermediate		Groups 1, 3, 4
Paraldehyde		8-12 c.c.	5-10	Intermediate	Liver and kidneys	Acute excitement delerium
Pentaerythritol chloral (Periclor)	0.3 Gm.	0.6-1.0 Gm.	15-20	Intermediate	Liver and kidneys	Groups 1, 2, 3, 4

*See text.

Table I *(Cont'd)*

Drug	*Sedative dose*	*Hypnotic dose*	*Usual onset of action (min.)*	*Duration*	*Metabolism*	*Usually best therapeutic uses*
Pentobarbital (Nembutal)	30 mg.	0.1 Gm.	20-30	Intermediate	Liver and kidneys	Groups 1, 2, 3, 4
Probarbital (Ipral)	0.13-0.26 Gm.	0.26-0.39 Gm.	20-30	Intermediate	Liver and kidneys	Groups 2, 3, 4
Phenobarbital	15-30 mg.	0.1 Gm.	20-40	Long	Liver and kidneys	Groups 3, 4
Secobarbital (Seconal)	15-30 mg.	0.1 Gm.	20-30	Short	Liver	Groups 1, 3
Talbutal (Lotusate)	30-50 mg.	0.12 Gm.	20-30	Intermediate	Liver and kidneys	Groups 2, 3, 4
Vinbarbital (Delvinal)	30 mg.	0.1-0.2 Gm.	20-30	Intermediate	Liver and kidneys	Groups 2, 3, 4

Therapeutic uses

Those exerting an immediate action which is quickly inactivated are of most use in treating patients in group 1, since their need is for aid in relaxing and falling to sleep after which they have no need for drug. Secobarbital and ethinamate are most useful in this type of insomnia. Chloral hydrate, although exerting a longer duration of action, is also useful in these patients because of its quick onset of action and its low toxicity and cost.

Sedatives with a slower onset of action but which exert a longer hypnotic effect are most useful for patients in groups 3 and 4. These patients need aid in remaining asleep. Frequently, sedatives must be combined with other agents if these individuals are to get relief from the insomnia. Pain from muscles, joints, or nerves may require analgesic, muscle-relaxing drugs or proper support for relief. The menopausal woman may only get sleep when her sweats are controlled by adequate estrogen therapy. The tense, psychologically upset insomnia patient may need the extra support of a potent tranquilizing drug before he can secure sleep.

The longer acting agents are useful in groups 2, 3, and 4. Frequently chloral hydrate and phenobarbital and occasionally barbital prove to be the drug of choice for these patients. Unfortunately, the cerebral depressant properties of phenobarbital may cause agitation, excitement, or confusion in the older, arteriosclerotic patient so often found in group 2. Many physicians will not use phenobarbital in their older patients for this reason. In the patient with Parkinson's disease, it is also likely to make the condition worse.

Barbital is not used much at the present. It seems to have a cumulative action. In older patients with limited kidney reserve, there is a distinct possibility that in many instances the somnolence experienced the day after an evening dose of barbital is due to impaired ability to eliminate the drug.

If, however, barbital is given in the soluble form at least 1 hour before sleep is expected and the dose adjusted to the patient's needs, often one-half the usual dose, it can be surprisingly effective for group 2 patients.

Chloral hydrate is highly useful in group 2, 3, and 4 patients and is well tolerated by the older individuals. Very seldom are reactions experienced to this drug, and there is a wide margin of safety in dose adjustment.

Older patients in group 2 frequently are awakened by aching leg muscles and joints or uncomfortable feelings in the legs, usually from poor circulation. A dose of acetylsalicylic acid or quinine sulfate given with the sedatives at bedtime may prove most helpful in controlling their insomnia.

Untoward effects

Very little in the way of toxic effects is seen when this class of agents is used wisely. Skin rashes are seen rarely with phenobarbital, glutethimide, and methyprylon and then are not usually serious. Only very rarely are fixed drug eruptions, more serious skin lesions, or blood or liver complications seen.

The chief difficulty in the uses of these agents arises when they are prescribed unwisely. Giving them without proper diagnostic study or without adequate control frequently leads to trouble. Prescribing excessive doses or being careless in permitting the patient to increase the dosage above safety levels is another cause of difficulty. When the dose is kept at the recommended levels (see Table I), addiction and dependence will seldom be seen. But if patients are permitted to increase their intake three or four times the usual dose and continue this for several months, many will develop severe physical dependence.

The sudden withdrawal of barbiturates and perhaps of glutethimide can precipitate a serious reaction if the patient has long been on large doses. Convulsions, excitement, agitation, and psychotic behavior have all been seen. Any patient who is suspected of or definitely does have physical dependence should be hospitalized and withdrawal gradually accomplished.

Finally, it is surprising how often an old-fashioned sedative like phenobarbital, chloral hydrate, or butabarbital is effective or even superior in action to the newer, expensive tranquilizers for the relief of tension states.

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